



BI
Biological Industries
Culture of Excellence

CATALOGUE

Cell Culture



Dear Customer,

The aim of our new catalogue is to introduce you to Biological Industries' cell culture related products, and manufacturing services. Our main mission is to provide you with products and services to maintain your cell culture under optimal growth conditions. Over 30 years experience of constant innovation together with regulatory and performance challenges enable us to continue making products that you may rely on and provide you with service you can trust. Please note that all our prices are very reasonable. In this catalogue you will find hundreds of cell culture-related products and complementary services that should provide one-stop-shopping solutions for your cell culture needs.

Biological Industries' Team

Advance your stem cell applications...

with a **complete, xeno-free hMSC culture system**

See pages 9-13



Product Name	Cat No.	Unit Size	Product Name	Cat No.	Unit Size
MSC NutriStem® XF Basal Medium	05-200-1A	500ml	Recombinant Trypsin Solution	03-078-1A	500ml
	05-200-1B	100ml		03-078-1B	100ml
MSC NutriStem® XF Supplement Mix	05-201-1U	3ml	Recombinant Trypsin-EDTA Solution	03-079-1A	500ml
	05-201-1-06	0.6ml		03-079-1B	100ml
MSC Dissociation Solution, Plant-Derived Enzyme	03-075-1B	100ml	MSC Freezing Solution	05-712-1D	10ml
	03-075-1C	20ml		05-712-1E	50ml
MSC Dissociation Solution, Non-Enzymatic	03-077-1A	500ml	MSC Attachment Solution	05-752-1S	0.1ml
	03-077-1B	100ml			
	03-077-1C	20ml			

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Customize your own formulation,
Start small think big...

Small volumes, upscaling capabilities, fast
turnaround and flexible packaging, tailor-made by
dedicated, experienced cell culture professionals

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Stem Cells

Human Embryonic Stem Cells and iPS cells
Mouse Embryonic Stem Cells
Human Adult (Mesenchymal) Stem Cells

1 Stem Cells

Human Embryonic Stem Cells and iPSCs

Human embryonic stem cells (hESCs) are pluripotent cells isolated from blastocysts. Traditionally, these cells have been cultured with a supporting layer in two-dimensional culture, which allows their continuous growth as undifferentiated cells. However, any future use of hESCs for cell-based therapy and industrial purposes will require a scalable, reproducible and controlled process. To this end, a defined serum- and xeno-free medium was developed. The medium was found to support feeder layer free and feeder-dependent culture of both embryonic and induced pluripotent stem cells.

NutriStem® hESC XF

Product Name	Catalogue No.	Unit Size	Storage Temp.
NutriStem® hESC XF Xeno-free medium for hESCs and iPSCs, With HSA, Optimized for feeder-free and feeder-dependent culture systems	05-100-1A	500ml	-20°C
	05-100-1B	100ml	-20°C
AF NutriStem® hESC XF Xeno-free medium for hESCs and iPSCs, Without HSA, Optimized for feeder- dependent culture systems	05-102-1A	500ml	-20°C
	05-102-1B	100ml	-20°C

Fully-defined, serum-free (SF) and xeno-free (XF) media, designed for the growth and expansion of Human Embryonic Stem Cells (hESC) and induced pluripotent stem cells (iPSC).

NutriStem® hESC XF media were developed in corporation with a leading group in stem cell research, to enable the maintenance and expansion of both hESCs and iPSC on feeder cells or in feeder-free culture system. NutriStem® hESC XF support the growth of undifferentiated pluripotent stem cells (PSC) on Matrigel, mouse feeder cells (MEF) or human foreskin fibroblasts (HFF). (See Figure 1). The media contain recombinant human basic fibroblast growth factor (rh bFGF) and recombinant human transforming growth factor β (rh TGF β). The media have been successfully tested and proven to maintain the pluripotential nature of hESCs.

Features

- A complete ready-to-use formulations (no additions are required).
- Contain Alanyl glutamine.
- Serum-free and xeno-free.
- Enable expansion of hESCs and iPSCs in feeder-free culture system (Matrigel™), human-feeder cells (foreskin fibroblasts) or on mouse feeder cells (MEFs).
- Support long-term growth of hESCs (over 50 passages).

- Extensively tested and proven to maintain robust pluripotency using several hESC lines including H1, H9.2, I6, I3.2, CL1.
- Maintain differentiation capability.
- Maintain normal phenotype (colony morphology) and genotype (karyotype) of hESCs.
- Low proteins, low b-FGF concentration.
- Provide gene expression profiles comparable to classical media.
- Intended for use in a 5% CO₂ atmosphere.
- Contain only the most essential components required for maintenance of hESC and iPSC cells, providing a simplified medium formulations.

Storage and Stability

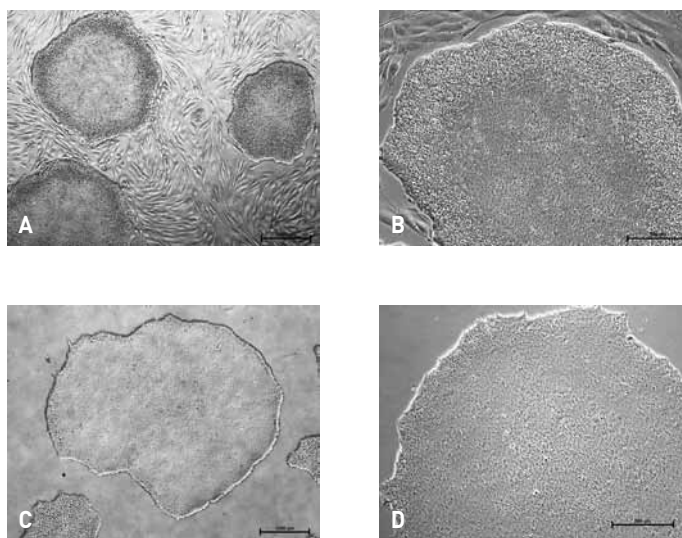
NutriStem® hESC XF should be stored at -20°C. Upon thawing, the medium may be stored at 2-8°C for 2 weeks. Dispense into aliquots to avoid repeated freezing and thawing.

Protect the medium from light.

Quality Control

NutriStem® hESC XF performance is tested for optimal maintenance and expansion of undifferentiated hESCs (H1 cell line). Additional standard evaluations are pH, osmolality, endotoxins, mycoplasma and sterility tests.

Figure 1: H1 hESCs cultured in NutriStem® hESC XF medium on MEF feeder layer (A,B) or Matrigel™ (C,D).



Human Serum Albumin (HSA)

Product Name	Catalogue No.	Unit Size	Storage Temp.
Bio-Pure Human Serum Albumin (HSA Solution, 10%)	05-720-1B	100ml	2-8°C
	05-720-1C	20ml	2-8°C

Bio-Pure HSA is specially optimized and qualified for the growth and expansion of undifferentiated pluripotent human ES and iPS cells, in both feeder-dependent and feeder-free conditions.

HSA is a medium supplement that is a highly soluble osmolytic protein with a high molecular weight. The primary function of HSA is not only its capability of binding anionic, cationic and neutral molecules, but it also has the proclivity of sequestering and stabilizing a wide array of ions and other small molecules.

Bio-Pure HSA is prepared from clinical-grade HSA approved for intravenous administration by the FDA and/or the EMEA.

All individual donations of the plasma and the corresponding plasma pool, are tested for Hepatitis B Surface Antigen (HBsAg), Anti (Human Immunodeficiency Virus) HIV-I and II and anti-HCV.

CryoStem- Cryopreservation medium

Product Name	Catalogue No.	Unit Size	Storage Temp.
CryoStem	05-710-1E	50ml	2-8°C
	05-710-1D	10ml	2-8°C

Animal component-free, protein-free and chemically defined freezing medium, for cryopreservation of human ES & iPS cell clumps.

There are many problems associated with the use of animal sera e.g. contamination with viral agents such as BSE, Hepatitis, HIV, BVD, or other potential adventitious agents. The culture of cells in animal component-free medium eliminates those risks. Furthermore, it allows cells to be grown under a defined set of conditions. When using serum-free media in cell culture, it is important to cryopreserve cells also in a medium free of serum.

The novel animal component-free human ES cell freezing medium that has been developed by Biological Industries contains no serum, but rather methylcellulose and DMSO. After freezing and thawing, a very high percentage of viable cells are obtained. In fact, comparative studies have shown that in most cases upon thawing and plating, higher viability and adhesion percentages are obtained in comparison to other freezing medium formulations including serum containing formulation. The use of CryoStem is also recommended for cryopreservation of cells cultured with serum containing media.

Features

- Chemically defined.
- Animal components- free (ACF).
- Protein-free.
- For freezing of human ES and iPS cells cultured in both feeder-free and feeder dependent systems.
- High recovery efficiency: maintains excellent attachment ability as well as growth performance.
- Maintains human ES and iPS cell pluripotency.

Mouse Embryonic Stem Cells

Mouse ES Cell Basal Medium

Product Name	Catalogue No.	Unit Size	Storage Temp.
Mouse Embryonic Stem Cell (ESC) Basal Medium , With L-Alanyl L-Glutamine	01-171-1A	500ml	2-8°C
	01-171-1B	100ml	2-8°C

Basal medium designed for the growth of mouse embryonic stem (ES) cells.

Mouse embryonic stem (ES) cells are pluripotent cells derived from the inner cell mass of the blastocyst. Undifferentiated ES cells can be maintained in-vitro for extended periods without loss of their capacity to differentiate to all cell lineages when reimplanted back into a blastocyst. ES cells may differentiate in-vitro to a variety of cell types including neuronal, muscle, endothelial and hematopoietic progenitors. General culture conditions are well established and usually require ES cells to be grown on an inactive feeder cell layer or on gelatin-coated plates with Leukemia Inhibitory Factor (LIF) in the culture medium. Mouse ES Basal Medium has been optimized to grow and maintain undifferentiated mouse embryonic stem cells. The medium may be used with the addition of Foetal Bovine Serum (FBS) or with any serum replacement designed for mouse ES cells.

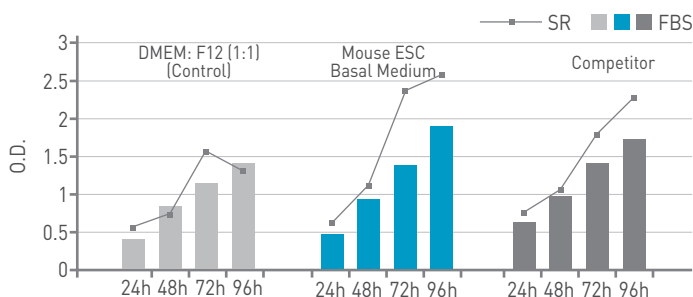
The medium contains L-Alanyl L-glutamine (stable glutamine).

Storage

Mouse ES Basal Medium should be kept at 2-8°C. Protect the medium from light.

Graph 1: Growth rate of mouse ESC (ES-D3) using Biological Industries' Mouse ES Basal Medium vs. a competitor's medium.

mESC were cultivated in a 96 well plates and observed over a period of 5 days. Proliferation rate was measured using a colorimetric method (XTT based Cell Proliferation Kit, Cat. No. 20-300-1000). Absorbance was read after 4 hours of incubation (wavelength of 450nm and reference of 690nm), proceeding addition of the XTT reagent.



Gelatin Solution, 0.1%

Product Name	Catalogue No.	Unit Size	Storage Temp.
Gelatin Solution (0.1%)	01-944-1A	500ml	AMB
	01-944-1B	100ml	AMB

Qualified for Mouse Embryonic Stem (ES) Cells.

Gelatin solution (0.1%) is intended for coating cell culture flasks or plates used for the growth of mouse ES cells without feeder layer and with the addition of LIF to the culture medium.

Human Adult (mesenchymal) stem cells

Introduction

Human MSC are multipotent cells with the ability to differentiate into cells of connective tissue lineages, including mainly adipocytes, osteoblasts and chondrocytes.

In addition, numerous recent studies have demonstrated the plasticity of hMSC. Thus, under appropriate culture conditions, hMSC can also differentiate into cells of endodermal and ectodermal.

hMSC have advantages over other stem cells types, due to the broad variety of their tissue sources and for being immuno-privileged. These traits have led hMSC to become desirable tools in regenerative medicine and cell therapy. Application of hMSC in cell therapy needs the elaboration of appropriate culture media and defined culture conditions in order to minimize the health risk of using xenogenic compounds and to limit the immunological reactions once MSCs are transplanted while maintaining multi-potentiality, self-renewal, and transplantability.

A Xeno-Free Culture System for Human Mesenchymal Stem Cells (hMSC)

To date, the most common culture media for growth and expansion of hMSC include animal components. In addition, the common auxiliary medium supplements and solutions (for attachment, freezing and dissociation) required for long term growth and maintenance of hMSC, are mostly animal-derived.

BI has developed a novel, serum-free and xeno-free culture system, for the growth and expansion of hMSC from variety of sources (bone marrow, adipose tissue and cord tissue).

The xeno-free culture system includes solutions for attachment, dissociation and cryopreservation of hMSCs, as well as, serum- and xeno-free culture medium for long-term growth of hMSCs, while maintaining their multi-lineage differentiation potential.

MSC NutriStem® XF Medium

Product Name	Catalogue No.	Unit Size	Storage Temp.
MSC NutriStem® XF Basal Medium	05-200-1A	500ml	2-8°C
MSC NutriStem® XF Supplement Mix	05-201-1U	3 ml	-5 to -20°C
MSC NutriStem® XF Basal Medium	05-200-1B	100 ml	2-8°C
MSC NutriStem® XF Supplement Mix	05-201-1-06	0.6 ml	-5 to -20°C

A Defined, Serum-Free (SF), Xeno-Free (XF) Medium, Designed to Support the Growth of hMSCs.

MSC NutriStem® XF Medium is a serum-free, xeno-free medium formulation developed for the growth and expansion of human mesenchymal stem cells after being isolated from a variety of sources (i.e., bone marrow, adipose tissue and umbilical cord tissue; hMSC-BM, hMSC-AT, hMSC-UCT). (See Figure 2). MSC NutriStem® XF Medium supports long-term growth of hMSCs while maintaining their self-renewal and multi-lineage differentiation potential. MSC NutriStem® XF Medium is recommended for use with MSC Attachment Solution (Cat. No. 05-752-1) for optimal attachment and spreading of cells. For optimal cell passage and long term culture of the cells, it is recommended to use the MSC Dissociation Solution (Cat. No. 03-075-1) or Recombinant Trypsin Solution (Cat. No. 03-078-1, 03-079-1)

Notes: No additional additives are required for the complete, ready-to-use medium. Components are not sold separately.

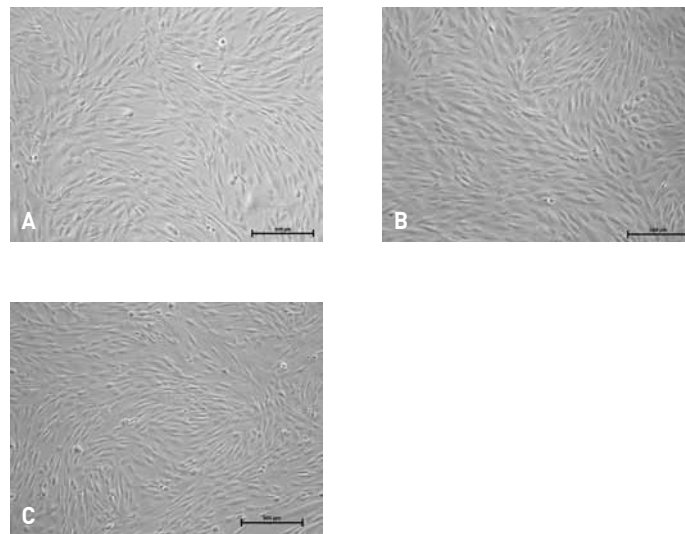
Features

- Serum-free (SF), xeno-free (XF) medium: all components are defined and from non-xenogenic origin.
- Does not contain antibiotics.
- Contains L-glutamine.
- Enables culture of hMSCs from various sources.
- Supports long-term growth of hMSCs, retaining the fibroblast-like cell structure.
- No background differentiation.
- Maintains hMSC self-renewal and multi-lineage differentiation potential (e.g. to osteocytes, adipocytes and chondrocytes).
- Human MSC cultured with MSC NutriStem® XF express high percentage of MSC surface markers and do not express hematopoietic markers.

Quality Control

MSC NutriStem® XF Medium performance is tested for optimal maintenance and expansion of undifferentiated hMSCs, while maintaining their multi-lineage differentiation potential. Additional tests are: pH, osmolality, endotoxins and sterility tests.

Figure 2: hMSCs cultured in MSC NutriStem® XF medium, 3 days post seeding. A. hMSC-AT; B. hMSC-BM and C. hMSC-UCT.



MSC Attachment Solution

Product Name	Catalogue No.	Unit Size	Storage Temp.
MSC Attachment Solution	05-752-1S	0.1ml	2-8°C
	05-752-1F	1ml	2-8°C
	05-752-1H	5ml	2-8°C

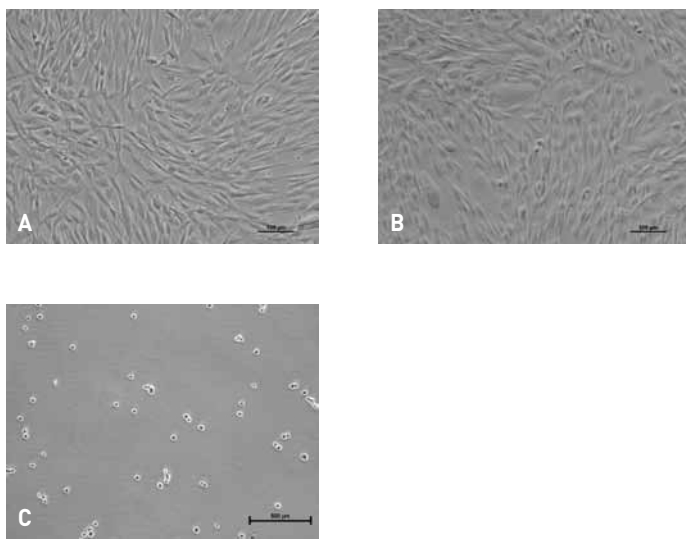
A xeno-free (XF) solution for facilitating attachment and spreading of hMSC in serum-free culture system.

MSC Attachment Solution is a clear sterile solution containing human fibronectin (hFN) obtained by affinity purification on gelatine-sepharose from human plasma. This solution is particularly useful for the culture of cells that are not capable of synthesizing their own biomatrix or when culturing cells in serum-free medium. MSC Attachment Solution was qualified and optimized for the attachment and spreading of human mesenchymal stem cells (hMSCs) when cultured in MSC NutriStem® XF culture medium (Cat. No. 05-200-1 and 05-201-1). (See Figure 3).

Features

- Xeno-free.
- Qualified for various sources of hMSCs (such as bone marrow, adipose tissue, umbilical cord tissue).
- Optimized for the attachment of hMSCs to tissue culture dishes when cultured with MSC NutriStem® XF medium.

Figure 3: hMSC-AT cultured on pre-coated plates with various attachment solutions in xeno-free medium. A. MSC Attachment Solution (BI cat # 05-752-1); B. CellStart (Invitrogen cat # A10142-01) and C. Without any attachment factor.



MSC Freezing Solution

Product Name	Catalogue No.	Unit Size	Storage Temp.
MSC Freezing Solution	05-712-1D	10ml	2-8°C
	05-712-1E	50ml	2-8°C

A chemically defined, animal component-free and protein-free formulation for optimal cryopreservation of hMSCs.

When culturing cells under serum free conditions, it is important to cryopreserve the cells also in a solution free of serum. The novel MSC Freezing Solution contains no serum, but rather methylcellulose and DMSO. After freezing and thawing, a very high percentage of viable hMSCs are obtained with excellent attachment ability as well as growth performance. In fact, comparative studies have shown that in most cases higher viabilities and adhesion percentages are obtained in comparison to serum-containing formulations. The use of this serum-free MSC Freezing Solution is also recommended for cryopreservation of cells cultured with serum-containing media.

Features

- A complete, ready-to-use solution.
- Protein-free.
- Without animal or human derived components.
- Suitable for various sources of hMSCs (such as bone marrow, adipose tissue, umbilical cord tissue).
- High cell viability after thawing.
- Suitable for hMSC cultured in both serum-containing and serum-free media.
- Optimized for hMSCs cultured in MSC NutriStem® XF Medium.

Recombinant Trypsin Solution

Product Name	Catalogue No.	Unit Size	Storage Temp.
Recombinant Trypsin Solution	03-078-1B	100mg	-20°C
	03-078-1A	500mg	-20°C
Recombinant Trypsin-EDTA Solution	03-079-1B	100mg	-20°C
	03-079-1A	500mg	-20°C

Special animal components-free (ACF) recombinant trypsin solutions, developed as an alternative to porcine trypsin. The solutions do not contain any chymotrypsin, carboxypeptidase A, and other protease contaminant.

Recombinant Trypsin Solution formulations were developed for efficient dissociation of adherent cell types from surfaces and tissues, and are optimized for sensitive cells, such as primary human mesenchymal stem cells (hMSC). The addition of EDTA usually accelerates the dissociation. Recombinant Trypsin solutions are pure enzyme solutions, which help maximize the yield of functionally viable cells from culture vessels, while preventing the toxicity effect induced by other non desirable proteases. In addition, recombinant trypsin eliminates the risk of viruses, or other potential adventitious agents found in animal derived components.

Features

- Ready-to-use.
- Non-animal or human origin.
- Increased specificity.
- Eliminates contaminating activities found in bulk production of enzymes.
- Free from undesirable proteases such as carboxypeptidase A and chymotrypsin.
- Optimized for hMSCs in both serum-containing and serum-free culture systems.

MSC Dissociation Solution (Plant-Derived Enzyme)

Product Name	Catalogue No.	Unit Size	Storage Temp.
MSC Dissociation Solution, Plant-Derived Enzyme	03-075-1B	100ml	-20°C
	03-075-1C	20ml	-20°C

An animal component-free cell dissociation solution, designed for rapid and efficient dislodging of human mesenchymal stem cells (hMSCs) from culture vessels.

To date, several commercial trypsin replacement solutions are available but not all of them are validated for hMSC.

MSC Dissociation Solution is based on a special plant-derived enzyme optimized for effective passaging of hMSCs cultured with MSC NutriStem® XF Medium (Cat. No. 05-200-1 and 05-201-1).

MSC Dissociation Solution is also suitable for hMSC cultured in serum-containing medium.

Features

- Ready-to-use.
- Without any animal or human derived components.
- Contains enzyme derived from a plant.
- Optimized for passaging hMSC cultured in MSC NutriStem® XF medium.
- Qualified for various sources of hMSCs (such as bone marrow, adipose tissue, umbilical cord tissue).
- Works with serum-free and serum-containing media.
- Allows rapid detachment together with high viability of cells.

MSC Dissociation Solution (Non-Enzymatic)

Product Name	Catalogue No.	Unit Size	Storage Temp.
MSC Dissociation Solution, Non-Enzymatic	03-077-1A	500ml	2-8°C
	03-077-1B	100ml	2-8°C
	03-077-1C	20ml	2-8°C

A chemically defined, animal component-free cell dissociation solution, for gentle and efficient dissociation of hMSCs, when used in conjunction with serum-free or serum-containing media.

MSC Dissociation Solution is a ready to use non-enzymatic solution formulated with a proprietary mixture of chelators for gentle dislodging adherent hMSC from culture vessels. Cell Dissociation Solution helps maximizing the yield of functionally viable cells. It is designed to be an alternative to enzyme based solutions when used in conjunction with serum-free or serum-containing media. Cells can be exposed to the Non-Enzymatic Cell Dissociation Solution for longer periods of time without the risk of damage associated with protein digestive enzymes like trypsin.

Features

- Chemically defined reagent contains a proprietary mixture of chelators.
- Ready-to-use.
- Contains no products of animal origin.
- Contains no enzymes or proteases.
- Works with serum-free and serum-containing media.
- Reduces the risk of cell damage associated with enzyme based dissociation solutions.

Ordering Information

Product Name	Catalogue No.	Unit Size	Storage Temp.
Human Embryonic Stem Cells and iPS Cells			
NutriStem® hESC XF Xeno-Free, Serum-Free Medium for Human ES Cells and iPS Cells, With HSA	05-100-1A	500ml	-20°C
	05-100-1B	100ml	-20°C
AF NutriStem® hESC XF Xeno-Free, Serum-Free Medium for Human ES Cells and iPS Cells, Without HSA	05-102-1A	500ml	-20°C
	05-102-1B	100ml	-20°C
Bio-Pure Human Serum Albumin (HSA Solution, 10%), Optimized for Human Embryonic Stem Cells (hESC)	05-720-1B	100ml	2-8°C
	05-720-1C	20ml	2-8°C
CryoStem	05-710-1E	50ml	2-8°C
	05-710-1D	10ml	2-8°C
Human Adult (Mesenchymal) Stem Cells			
MSC NutriStem® XF Basal Medium	05-200-1A	500ml	2-8°C
	05-200-1B	100ml	2-8°C
MSC NutriStem® XF Supplement Mix	05-201-1U	3ml	-20°C
	05-201-1-06	0.6ml	-20°C
MSC Dissociation Solution, Plant-Derived Enzyme	03-075-1B	100ml	-20°C
	03-075-1C	20ml	-20°C
MSC Dissociation Solution, Non-Enzymatic	03-077-1A	500ml	2-8°C
	03-077-1B	100ml	2-8°C
	03-077-1C	20ml	2-8°C
Recombinant Trypsin Solution	03-078-1A	500ml	-20°C
	03-078-1B	100ml	-20°C
Recombinant Trypsin-EDTA Solution ¹	03-079-1A	500ml	-20°C
	03-079-1B	100ml	-20°C
MSC Freezing Solution	05-712-1E	50ml	2-8°C
	05-712-1D	10ml	2-8°C
MSC Attachment Solution	05-752-1H	5ml	2-8°C
	05-752-1F	1ml	2-8°C
	05-752-1S	0.1ml	2-8°C
Mouse Embryonic Stem Cell			
Mouse Embryonic Stem Cells (mESC) Basal Medium, with L-Alanyl L-Glutamine	01-171-1A	500ml	2-8°C
	01-171-1B	100ml	2-8°C
Gelatin Solution (0.1%)	01-944-1A	500ml	AMB
	01-944-1B	100ml	AMB
Foetal Bovine Serum Qualified for Stem Cells			
Certified Foetal Bovine Serum (FBS) Qualified for Human Embryonic Stem Cells	04-002-1A	500ml	-20°C
	04-002-1B	100ml	-20°C
Certified Foetal Bovine Serum (FBS) Qualified for Human Embryonic Stem Cells, Heat Inactivated	04-222-1A	500ml	-20°C
	04-222-1B	100ml	-20°C
Certified Foetal Bovine Serum (FBS) Qualified for Mesenchymal Stem Cells	04-400-1A	500ml	-20°C
	04-400-1B	100ml	-20°C

Product Name	Catalogue No.	Unit Size	Storage Temp.
Auxiliary Solutions			
Dulbecco's Modified Eagle Medium (DMEM): Nutrient Mixture F-12 (Ham's) (1:1) Without L-Glutamine With Sodium Bicarbonate 1.2gm/l With Hepes 15mM With Sodium Pyruvate 55mg/l	01-170-1A	500ml	2-8°C
	01-170-1B	100ml	2-8°C
MEM Non-Essential Amino Acids Solution 100X Conc.	01-340-1B	100ml	2-8°C
Human Recombinant Insulin Solution ~3.5 mg/ml	01-818-1H	5ml	2-8°C
Serum-Free Cell Freezing Medium	05-065-1A	500ml	2-8°C
	05-065-1C	20ml	2-8°C
L-Glutamine Solution 29.2mg/ml in Saline, 200 mM	03-020-1A	500ml	-20°C
	03-020-1B	100ml	-20°C
	03-020-1C	20ml	-20°C
L-Alanyl-L-Glutamine (Stable Glutamine) 200 mM	03-022-1B	100ml	-20°C
Sodium Pyruvate Solution 11.0mg/ml (100 mM)	03-042-1B	100ml	-20°C
Crystalline Trypsin Solution (0.02%) Without Phenol Red	03-047-1A	500ml	-20°C
	03-047-1B	100ml	-20°C
Soybean Trypsin Inhibitor 50X Conc 5mg/ml	03-048-1C	20ml	-20°C
Cell Dissociation Solution (non-enzymatic)	03-071-1B	100ml	2-8°C
Papain Dissociation Solution	03-072-1B	100ml	-20°C
Bovine Fibronectin Solution, 1mg/ml	03-090-1-01	1ml	2-8°C
	03-090-1-05	5ml	2-8°C
Human Fibronectin Solution, 1mg/ml	05-750-1F	1ml	2-8°C
	05-750-1H	5ml	2-8°C
Transferrin, Human Substantially Iron-Free (APO)	41-951-100	100mg	2-8°C
	41-951-500	500mg	2-8°C
Transferrin, Human Iron-Saturated (HOLO)	41-952-100	100mg	2-8°C
	41-952-500	500mg	2-8°C
Insulin, Human Recombinant	41-975-100	100mg	2-8°C
Basic Fibroblast Growth Factor (b-FGF)	30-T-218A	10µg	-20°C
	30-T-218B	50µg	-20°C

Vero Cells
Hybridoma Cells
Anchorage-Dependant Cells
CHO Cells
Mononuclear Cells (Immunology)
Insect Cells
Cryopreservation
Auxiliary Solutions

2 Serum-Free & Animal Component-Free Media & Reagents

Vero Cells

In the light of the growing worldwide shortage and consequent price instability associated with foetal bovine serum, the development of effective serum-free medium formulations has become essential for the future growth of the biotechnology industries. Batch variation in FBS requires prior sampling of each lot. Also the use of FBS in production of biologicals causes downstream purification difficulties.

NutriVero™ VP

Product Name	Catalogue No.	Unit Size	Storage Temp.
NutriVero™ VP1, Animal Component-Free Serum-Free Medium for the Monolayer Culture of Vero Cells (NutriVero™ VP1, ACF SFM)	05-066-1A	500ml	2-8°C
	05-066-1B	100ml	2-8°C
NutriVero™ VP2, Animal Component-Free Serum-Free Medium for the Microcarrier Suspension Culture of Vero Cells (NutriVero™ VP2, ACF SFM)	05-067-1A	500ml	2-8°C
	05-067-1B	100ml	2-8°C

A chemically defined, animal and human component-free serum-free medium, designed to support the growth of Vero cells used in virology, virus production, and biotechnology.

There are many problems associated with the use of animal sera e.g. the fear of contamination with viral agents such as BSE, Hepatitis, HIV, BVD or other potential adventitious agents. The culture of cells in serum-free and animal component-free medium eliminates those risks. Furthermore, it allows cells to be grown under a defined set of conditions.

NutriVero™ VP1 and NutriVero™ VP2 are serum free, very low protein media containing no proteins or peptides of human or animal origin. **NutriVero™ VP1** - designed specifically for monolayer culture of Vero cells.

NutriVero™ VP2 - designed specifically for microcarriers suspension culture of Vero cells. NutriVero™ VP1 and NutriVero™ VP2 are both suitable for large scale culturing and for growing viruses, as well as other cell culture applications, including production of recombinant proteins. The medium contains EGF and does not contain L-glutamine.

Features

- Very low protein concentration.
- No proteins or peptides of animal or human origin.
- The proteins that are used are human recombinant EGF and human recombinant Insulin.
- The formulation is without any animal origin components.
- Reduced risk of viral contamination.
- Lot to lot consistency.
- Ease of downstream product purification.

Quality Control

NutriVero™ VP1 and NutriVero™ VP2 are performance tested using Vero cells pre-adapted to serum-free culture in NutriVero™ VP1 and NutriVero™ VP2 correspondently. Additional standard evaluations are pH, osmolality and sterility tests.

Figure 1: Growth of Vero cells with NutriVero™ VP2 in microcarriers suspension culture (Cytodex-1) in spinner flask; cell counting performed using crystal violet nuclei staining method.

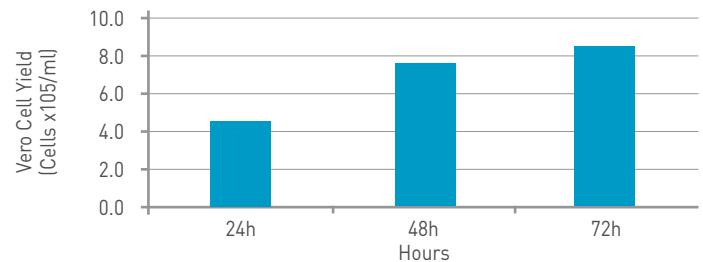
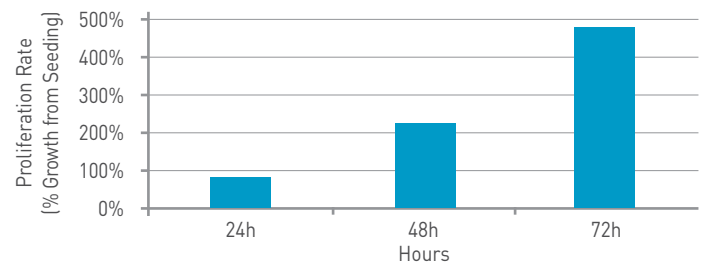


Figure 2: Growth of Vero cells with NutriVero™ VP2 in Bioreactor.



Hybridoma Cells

Biological Industries offers a series of serum-free media products for the growth of cells in suspension.

DCCM-1, DCCM-2, LPM, BIOGRO-1 & BIOGRO-2

Product Name	Catalogue No.	Unit Size	Storage Temp.
DCCM-1 Without L-Glutamine	05-010-1A	500ml	2-8°C
	05-010-1B	100ml	2-8°C
DCCM-1 10X Conc. Without L-Glutamine Without Sodium Bicarbonate	05-010-5B	100ml	2-8°C
DCCM-2 Without L-Glutamine	05-015-1A	500ml	2-8°C
	05-015-1B	100ml	2-8°C
DCCM-2 10X Conc. Without L-Glutamine, Without Sodium Bicarbonate	05-015-5A	500ml	2-8°C
	05-015-5B	100ml	2-8°C
Low Protein Media BSA-Free (LPM) Without L-Glutamine	05-040-1A	500ml	2-8°C
	05-040-1B	100ml	2-8°C
Low Protein Media BSA-Free (LPM) 10X Conc. Without L-Glutamine Without Sodium Bicarbonate	05-040-5B	100ml	2-8°C
BIOGRO-1 Serum-Free Medium Supplement 50X Conc.	05-600-1B	100ml	-20°C
	05-600-1C	20ml	-20°C
	05-600-1D	10ml	-20°C
	05-600-1T	2ml	-20°C
BIOGRO-2 Serum-Free Medium Supplement 50X Conc.	05-610-1B	100ml	-20°C
	05-610-1C	20ml	-20°C
	05-610-1D	10ml	-20°C
	05-610-1T	2ml	-20°C

Applications

These formulations have been successfully used in all of the following cell culture applications:

- Culture of myeloma and hybridoma cells.
- Monoclonal antibody production.
- Culture of human lymphocytes cells (including stimulated or transformed cells).

The formulations of DCCM-1 and DCCM-2 contain no growth factors and are therefore cost efficient. The relatively higher protein content of DCCM-1 is aimed at maximizing cell growth, while the lower protein content in DCCM-2 represents a compromise between cell growth promotion and easier purification in monoclonal antibody production.

LPM Medium is a formulation totally free of bovine serum albumin. The protein content is therefore less than 18 micrograms per ml. Despite this very low protein content LPM has proven to be very effective for the growth of a wide variety of hybridomas and other lymphocytes.

BIOGRO-1 and BIOGRO-2 are serum-free supplements intended for those customers who prefer to prepare their own final medium using a basal medium of their choice.

Method of Use

DCCM-1, DCCM-2 and LPM are ready-to-use media. They only require the addition of L-Glutamine and antibiotics.

The BIOGRO products are 50 fold concentrates and therefore are recommended for use at a concentration of 2% (although in some cases 1% may be sufficient). DMEM: F-12 (1:1) has been found to be most generally effective as the basal media, but many cell lines grow well with RPMI, DMEM or Iscove's. Glutamine and antibiotics should be added to the final formulation.

Adaptation of Cells

For many cell types no adaptation procedures are necessary and may even be detrimental. In other cases standard-weaning procedures may be necessary.

Recommended Amounts of Sodium Bicarbonate and L-Glutamine to be Added in the Preparation of Single Strength Liquid Media (1x) from Concentrated Media (10x)

Product Name	Desired Product (1x) Cat. No.	Prepared from Product (10x) Cat. No.	Quantity Sodium Bicarbonate Solution 7.5% Cat. No. 03-040-1 ml/Liter	Quantity L-Glutamine Solution 200mM Cat. No. 03-020-1 ml/Liter
DCCM-1	05-010-1	05-010-5	29.4	10-20
DCCM-2	05-015-1	05-015-5	29.4	10-20
Low Protein Media BSA-Free (LPM)	05-040-1	05-040-5	29.4	10-20

Anchorage-Dependant Cells

A successful transition from cell culture work utilizing serum-containing media to serum-free cell culture often requires the use of techniques which were specifically developed for this purpose. For example, special techniques for trypsinization, neutralization of trypsin, cryopreservation of cells, as well as the use of an effective serum-free growth medium are all essential.

BIO-MPM-1, BIOCHO-1, BIOCHO-2 and BIOGRO-CHO have been successfully used in adherent and suspension cultures.

BIO-MPM-1 Multi-Purpose Serum-Free Medium

Product Name	Catalogue No.	Unit Size	Storage Temp.
BIO-MPM-1, Multi-Purpose SFM Without L-Glutamine	05-060-1A	500ml	2-8°C
	05-060-1B	100ml	2-8°C

Bio-MPM-1 is a ready-to-use serum-free medium for adherent cells, after the addition of 2 mM glutamine. The formulation contains no albumin, which has been found to be non-essential for cell growth, and even prevents efficient adhesion in some cases. The protein content of BIO-MPM-1 is therefore less than 30mg per liter, and the medium contains no growth factors or hormones other than insulin. The formulation also contains no attachment factor, which in many (but not all) cases must be added for successful use.

Adaptation of Cells

In most cases it is possible to seed the cells that have been removed from freezing medium directly in BIO-MPM-1, when the cell concentration is at least 5×10^5 cells per 25cm^2 . The cells will begin to grow in BIO-MPM-1, and after a few passages the adaptation will be complete. However, in those cases where the cells do not adapt successfully after direct transfer, it will be necessary to perform gradual adaptation (weaning). The cells should be seeded with BIO-MPM-1 containing 5% serum and the serum concentration is then gradually reduced with each passage. The stage at which serum is completely removed is determined in the course of the weaning for each specific case. In order to save time, we recommend parallel experiments with direct adaptation and with weaning. Generally, after the first or second passage, it will be obvious whether direct adaptation has been successful, and if not, only the weaning experiments are continued. As part of these experiments it is also necessary to test for the possible requirement of the addition of fibronectin. After successful adaptation, it is recommended to cryopreserve the cells in Serum-Free Freezing Medium, in order to avoid the necessity of any further adaptation in the future.

Growth of Various Anchorage Dependent Cells in BIO-MPM-1 as Compared with Conventional Serum-Supplemented Medium⁽¹⁾

Cell	10% FBS			BIO-MPM-1		
	Seeding density/ cm^2	Doubling time (hours)	Maximum density/ cm^2	Additives	Doubling time (hours)	Maximum density/ cm^2
3T3	5×10^3	24.0	3.3×10^5	Bombesin SBTI ⁽³⁾ Fibronectin	25.2	3.3×10^5
A-549	1×10^4	26.4	4.5×10^5	----	33.0	2.8×10^5
B16-F10	5×10^3	30.0	5.0×10^5	----	30.0	5.5×10^5
BGM	1×10^4	19.2	4.0×10^5	Fibronectin	30.5	3.4×10^5
BHK-21	2.5×10^4	14.4	4.5×10^5	Fibronectin	12.0	9.0×10^5
BS-C-1	1×10^4	24.0	2.8×10^5	----	28.0	1.9×10^5
CEF	1.2×10^4	28.8	----	----	36.3	----
HELA	5×10^3	48.0	6.5×10^5	Fibronectin	36.0	6.0×10^5
HEp-2	5×10^3	57.0	5.5×10^5	Fibronectin	30.0	6.5×10^5
MA-10 ⁽²⁾	2.5×10^4	18.0	2.7×10^5	Fibronectin	16.5	3.8×10^5
VERO	5×10^3	16.5	4.1×10^5	Fibronectin	18.0	3.8×10^5

⁽¹⁾ MEM + 10% FBS: 3T3, A-549, BHK-21, BS-C-1, VERO.

RPMI-1640 + 10% FBS: B16-F10, BGM, HELA, HEp-2
M-199/F10 (1:2): CEF

⁽²⁾ Cells do not grow with FBS but with 15% horse serum in RPMI

⁽³⁾ Soybean trypsin inhibitor

Recommended Amounts of Sodium Bicarbonate and L-Glutamine to be Added in the Preparation of Single Strength Liquid Media (1x) from Concentrated Media (10x)

Product Name	Desired Product (1x) Cat. No.	Prepared from Product (10x) Cat. No.	Quantity Sodium Bicarbonate Solution 7.5% Cat. No. 03-040-1 ml/Liter	Quantity L-Glutamine Solution 200mM Cat. No. 03-020-1 ml/Liter
BIO-MPM-1, Multi Purpose SFM	05-060-1	05-060-5	26.9	10-20

CHO Cells

BIOCHO-1 Serum-Free Medium

Product Name	Catalogue No.	Unit Size	Storage Temp.
BIOCHO-1 Serum-Free Medium Base Without L-Glutamine	05-061-1A	500ml	2-8°C
	05-061-1B	100ml	2-8°C
BIOGRO-CHO Serum-Free Medium Supplement 100X Conc.	05-620-1E	50ml	-20°C
	05-620-1F	1ml	-20°C
	05-620-1H	5ml	-20°C

BIOCHO-1 SFM Base is the basic formulation for CHO cells. The solution contains amino acids, vitamins, salts, lipids and trace elements.

This medium is intended for the growth of CHO cells of various kinds: CHO-K1, and transfected cells containing recombinant DNA related to the DHFR gene.

BIOGRO-CHO SFM Supplement contains proteins and other components that require storage at -20°C. This product is a 100-fold concentrate. Preparation of the complete medium is carried out by adding 1% BIOGRO-CHO SFM Supplement to BIOCHO-1 SFM Base, and glutamine is then added.

The complete medium does not contain albumin, growth factors or hormones, other than insulin. Total protein concentration is less than 30mg per liter.

After preparation, the complete medium can be stored for up to 30 days at 2-8°C. Prolonged exposure to light should be avoided.

Adaptation of CHO Cells

In most cases it is possible to seed CHO cells that have been removed from freezing medium directly in the serum-free medium, when the cell concentration is at least 5×10^5 cells per 25cm^2 . The cells will begin to grow, and after a few passages the adaptation will be complete. However, in those cases where the cells do not adapt successfully after direct transfer, it will be necessary to perform gradual adaptation (weaning). The cells should be seeded with serum-free medium containing 5% serum and the serum concentration is then gradually reduced with each passage. The stage at which serum is completely removed is determined in the course of the weaning for each specific case.

In order to save time, we recommend parallel experiments with direct adaptation and with weaning. Generally, after the first or second transfer, it will be obvious whether direct adaptation has been successful, and if not, only the weaning experiments are continued.

After successful adaptation, it is recommended to cryopreserve the cells in Serum-Free Freezing Medium, in order to avoid the necessity of any further adaptation in the future.

Mononuclear Cells (Immunology)

BIOTARGET™-1 Serum-Free Medium

Product Name	Catalogue No.	Unit Size	Storage Temp.
BIOTARGET™-1 Without L-Glutamine	05-080-1A	500ml	2-8°C
	05-080-1B	100ml	2-8°C

BIOTARGET™-1 has been developed specifically for use with human mononuclear cells (lymphocytes and monocytes) from peripheral blood. In work with these cells and their sub-populations, it is critical to optimize and define the media formulation as well as pH and temperature.

In most cases, up until now, these cells are grown in conventional media, supplemented with human serum (A, AB) or foetal bovine serum. However, the use of serum suffers from the following disadvantages:

- The serum may contain non-specific growth factors, which interfere with complete activation in the desired direction.
- The serum may contain inhibitors which will limit activation of the lymphocytes.
- Lot to lot variation is certain.
- Pathogens may be introduced via the serum.
- The evaluation of the antigenic reaction, such as the quantity of the lymphokines generated, and the reaction of the lymphokines to hormones and growth factors are all more accurate in the absence of serum.

Applications for BIOTARGET™-1

The applications for the use of BIOTARGET™-1 are numerous and include:

1. Activation of mononuclear cells with the aid of various mitogens (PHA, CON.A, OKT-3).
2. Activation of mononuclear cells with lymphoid cells (RAJI, PEER, BA, MOLT-4, JURKAT).
3. Production of IL-2 and IL-3 from mononuclear cells.
4. Long-term culture of mononuclear cells after activation.
5. Activation of mononuclear cells with interleukin-2 in order to generate LAK or TIL cells.
6. Activation of mononuclear cells in order to generate natural killer cells (NK).
7. Activation of mononuclear cells in order to generate cytotoxic T cells.
8. Activation of macrophages.
9. Research on the influence of various cytokines on the production of sub-populations of mononuclear cells.
10. Proliferation of the HIV virus.
11. Proliferation of retroviruses in T cells for the purposes of vaccine development
12. Proliferation of retroviruses in T cells for the purposes of vaccine development

Following are several examples of the evaluation protocols by which BIOTARGET™-1 was selected:

1. Mitogenic Activation of Mononuclear Cells

Activation was evaluated with different mitogens such as PHA, CON.A and OKT-3. Proliferation was checked by measurement of the uptake of radioactive thymidine. The mitogens were added in varying concentrations and thymidine uptake was determined over several days, in order to fully evaluate the specific medium formulation.

2. Activation of Mononuclear Cells

with Lymphoid Cells The activation of the mononuclear cells was carried out using lymphoid cells of various kinds, such as: JURKAT, RAJI, MOLT-4, and BA. Varying ratios between the tumor cells and the mononuclear cells were examined, and the proliferation was checked by measurement of the uptake of radioactive thymidine.

3. Production of Lymphokines by Activated Mononuclear Cells

The levels of the lymphokines IL-2 and IL-3 were measured in the culture of the mononuclear cells after activation with various mitogens. IL-2 production was measured with the help of the CTLL-2 cell line. These are cytotoxic T-cells from mice, which grow only in the presence of IL-2 in the culture medium.

4. Cytotoxicity

Mononuclear cells were seeded at a concentration of 10^6 cells per well together with RAJI cells which had been treated with mitomycin C. Varying ratios of the two cell types were examined. At the conclusion of the activation (5-7 days), the lymphocytes were collected, centrifuged, suspended in medium and seeded in microwells in order to measure proliferation and cytotoxicity. RAJI cells were labeled with radioactive chromium (10 pCi in a volume of 0.2 ml), washed three times, suspended at a concentration of 105 cells per ml, and divided into microwells containing the above activated lymphocytes. After 18 hours incubation, the cytolytic activity was evaluated by measuring the radioactive chromium released from the target (RAJI) cells.

Insect Cells

BIOINSECT-1 Serum-Free Medium

Product Name	Catalogue No.	Unit Size	Storage Temp.
BIOINSECT-1, With L-Glutamine	05-050-1A	500ml	2-8°C

BIOINSECT-1 is a serum-free medium optimized for the culture of lepidopteran insect cells. The medium supports both suspension and stationary cultures of Sf-9 cells derived from the pupal ovarian tissue of *Spodoptera frugiperda*. Sf-9 cells are suitable hosts for the replication of the baculovirus *Autographa colifornica* nuclear polyhedrosis virus. This virus, isolated from the Alfalfa looper, is used for the recombinant expression of heterologous proteins in the baculovirus expression vector system (BEVS). Insect cells, infected with this virus, display accumulations of the highly expressed protein polyhedrin, within the nuclea (polyhedra). This protein-free medium supports the growth of Sf-9 cells with significantly better results than those obtained using TNM-FH medium (supplemented Grace's) with 10% foetal bovine serum, and production of recombinant beta-galactosidase is also excellent. BIOINSECT has showed excellent performance when cultivating high-V cells.

Weaning Procedure

Transfer cells in the logarithmic phase from the serum-containing medium into 50% (v/v) mixture of serum-supplemented medium and BIOINSECT-1.

Subculture the cells after 3 days and reduce the percentage of the serum-supplemented medium to 40%.

Continue with the subculturing of the cells every 3 days and with each passage reduce the concentration of the serum-supplemented medium by a further 10%. On the sixth passage, the cells will be fully adapted to BIOINSECT-1 serum-free medium.

Maintenance of Sf-9 cells in BIOINSECT-1 serum-free medium:

	Stationary culture	Suspension culture
Inoculation density	6-10 x 10^6 cells/cm ² 2-3 times/week	1.5 x 10^6 cells/ml Every 3-4 days
Subculture	Subculture the cells when the viable cell count reaches 4-5 x 10^5 /cm ² , with greater than 90% viability.	Subculture the cells when the viable cell count reaches 3-5 x 10^6 /ml, with greater than 95% viability. After 5 days in culture, the cell density reaches 6-8 x 10^6 cells/ml.

The culture may be gently centrifuged when subculturing, in order to remove the toxic by-products in the supernatant.

Cryopreservation

Serum-Free Cell Freezing Medium

Product Name	Catalogue No.	Unit Size	Storage Temp.
Serum-Free Cell Freezing Medium PF, ACF	05-065-1A	500ml	2-8°C
	05-065-1B	20ml	2-8°C

Protein-Free, Animal Component- Free (ACF)

When using serum-free media in mammalian cell culture, it is important to cryopreserve cells also in a medium free of serum. The novel cell freezing medium that has been developed by Biological Industries contains no serum, no proteins and no animal components but rather methylcellulose and DMSO. After freezing and thawing, a very high percentage of viable cells is obtained, and they also show excellent attachment ability as well as growth performance. In fact comparative studies have shown that in most cases higher viabilities and adhesion percentages are obtained in comparison to serum-containing freezing medium. Therefore, the use of this serum-free freezing medium is also recommended for cell culture employing serum-supplemented growth media.

Performance Validation

Serum-free Freezing Medium is a complete, ready to use solution which is designed to protect frozen cells in liquid nitrogen for long-term storage, without any use of protein or other animal components.

1. Materials and methods

1.1 Cell lines

Various cells grown under serum-free conditions were frozen with serum-free freezing medium and with freezing medium containing serum.

1.2 Freezing method

Serum-free Freezing Medium (Cat. no.: 05-065-1) and basal medium containing 10% DMSO and 20% FBS were used as freezing media. The cells were frozen in the appropriate freezing medium in a concentration of $3-5 \times 10^6$ per ml. One ml of these cell suspensions was transferred to a plastic ampoule and frozen by decreasing the temperature at a rate of 1-2°C/min. The ampoules were kept in liquid nitrogen until tested.

1.3 Cell recovery measurements

When thawing, the frozen ampoules were put in a water bath at 37°C. After dilution with culture medium and centrifugation, the cells were resuspended with either serum-free medium or medium containing serum. Viability of cells was determined by the trypan blue dye exclusion method. Adhesion of cells was determined by counting the attached cells only 6-24 hours after culture of the cells.

2. Results

2.1 Freezing of cells in Serum-free Freezing Medium in comparison to freezing medium containing serum:

Table 1: Thawing of cells 24 hours after freezing in liquid nitrogen

Cell	Viability %		Adhesion %	
	Serum-free Freezing Medium	Freezing Medium Containing Serum	Serum-free Freezing Medium	Freezing Medium Containing Serum
3T3	85	83	100	83
BGM	91	83	88	88
VERO	66	71	62	33
HEp-2	75	69	100	92
BSC-1	82	77	22	10

2.2 Long term storage of cells in liquid nitrogen using Serum-free Freezing Medium.

Table 2: Recovery of cells frozen in Serum-free Freezing Medium

Cell	24 hours storage		6 months storage		4 years storage	
	Viability	Adhesion	Viability	Adhesion	Viability	Adhesion
B16-F10	85	100	74	79	72	80
BGM	80	70	61	100	66	92
BHK-21	80	93	64	100	71	95
HELA	87	78	70	90	80	83
HEp-2	90	100	63	100	66	94
MA-10	88	95	81	100	83	91
VERO	90	94	71	68	73	76

3. Summary

The Serum-free Freezing Medium supports efficient cryopreservation of various cell lines cultured in serum-free media. After freezing and thawing, a very high percentage of viable cells is obtained, and they also show excellent attachment ability as well as growth performance. In fact, the present study has shown that in most cases higher viabilities and adhesion percentages are obtained in comparison to freezing medium containing serum. Therefore, the use of this Serum-free Freezing Medium is also recommended for cell culture employing serum-supplemented growth media.

Method of use

It is recommended to detach the adherent cells (to be frozen) with crystalline trypsin solution, and neutralization with Soybean Trypsin Inhibitor Solution. After centrifuging, suspend the cells in cold serum-free freezing medium at a concentration of 3-5 million cells per mL. Freeze the cells gradually (1-2°C per minute) and store them in liquid nitrogen. Thawing should be performed at 37°C. Immediately after thawing, suspend the cells in serum-free growth medium at a ratio of at least 1:10. Then centrifuge and suspend at high concentration in growth medium.

Auxiliary Solutions**Cell Dissociation Solution (Non-Enzymatic)**

Product Name	Catalogue No.	Unit Size	Storage Temp.
Cell Dissociation Solution (non-enzymatic)	03-071-1B	100ml	2-8°C

Cell Dissociation Solution is a special, non-enzymatic formulation with a proprietary mixture of chelators for gently dislodging adherent cell types from culture vessels. Cell Dissociation Solution helps to maximize the yield of functionally viable cells from these culture vessels. It is a non-enzymatic, protein-free and animal component-free solution. Another major advantage is that cells can be exposed to this solution for longer periods of time without the risk of subjecting them to protein digestive enzymes such as trypsin. However, the solution is not recommended for cells with very adhesive properties. For those cell lines which are difficult to dislodge, Biological Industries has developed a Papain Dissociation Solution.

Features

Contains a proprietary mixture of chelators. Contains no enzymes or proteases.

- Works with serum-free and serum-containing media.
- Reduces the risk of cell damage associated with trypsin.
- Chemically defined.
- Contains no products of animal origin.
- Supplied as a ready-to-use solution.

Papain Dissociation Solution

Product Name	Catalogue No.	Unit Size	Storage Temp.
Papain Dissociation Solution	03-072-1B	100ml	-20°C

Papain is a nonspecific, endolytic, sulfhydryl protease or protein-cleaving enzyme, known as cysteine-endopeptidase, and is derived and isolated from papaya fruit (i.e. *Carica papaya*). More specifically, it is isolated from the papaya latex, which is then utilized in a wide variety of applications. Papain is commonly used in cell isolation procedures, where it has proven to be more efficient and less destructive than other proteases on certain tissues such as and including, among others, the dissociation of retinal neurons⁽¹⁾, in the preparation of primary neurons from the visual cortex of postnatal rats⁽²⁾, and for the isolation of smooth muscle cells⁽³⁾.

Papain has a wide specificity in that it will degrade most protein substrates more extensively than the pancreatic proteases and has been proven not only to manifest fewer untoward and negative ramifications producing less cell and tissue trauma, but also to be much more effective than other available proteases. Biological Industries' Papain Dissociation Solution is a ready-to-use solution and is one of our non-animal alternatives for trypsin.

Physical Properties and Kinetics

Papain is a cysteine protease hydrolase enzyme of the peptidase C1 family derived from the papaya family, *Carica papaya* and the mountain papaya, *Vasconcellea cundinamarcensis*. It consists of a single peptide chain with three disulfide bridges and a sulfhydryl group necessary for the activity of the enzyme.

Specificity

Papain is more effective in digesting most protein substrates more extensively and effectively than pancreatic proteases. It further exhibits broad specificity cleaving peptide bonds of such basic amino acids as leucine and glycine. In addition to the aforementioned activity, it also hydrolyzes esters and amides.

⁽¹⁾ Shen J., et al., Japanese Journal of Physiology, 1995

⁽²⁾ Huettner, J.E. Baughman, R.W., Journal Of Neuroscience, 1986

⁽³⁾ Kinoshita, K. et.al., American Journal of Physiology, Gastrointestinal and Liver Physiology, 2003 and Driska, S.P. et.al., Journal of Applied Physiology, 1999.

Human Fibronectin Solution, 1mg/ml

Product Name	Catalogue No.	Unit Size	Storage Temp.
Human Fibronectin Solution, 1mg/ml	05-750-1H	1ml	2-8°C
	05-750-1F	5ml	2-8°C

Human Fibronectin (hFN) was tested and found suitable matrix for many cell types as well as for stem cells (e.g. mesenchymal stem cells). Biological Industries hFN is obtained by affinity purification on gelatine-sepharose from human plasma.

Features

- A complete ready-to-use solution.
- Suitable for various animal cells.
- Performance tested.

Bovine Fibronectin Solution, 1mg/ml

Product Name	Catalogue No.	Unit Size	Storage Temp.
Fibronectin Solution (Bovine), 1mg/ml	03-090-1-01	1ml	2-8°C
	03-090-1-05	5ml	2-8°C

Fibronectin is an attachment factor that facilitates the attachment and cytoplasmic spreading of all types of anchorage-dependent cells. Fibronectin is particularly useful for the culture of cells that are not capable of synthesizing their own biomatrix, or when culturing cells in serum-free medium.

Suggested Coating Procedures

The Fibronectin should be added to the growth medium in the growth vessel, which is then placed in an incubator 30-60 minutes before seeding. The recommended concentration of the Fibronectin is 5 micrograms per ml of medium. When the medium is replaced in the days following initial seeding, no further Fibronectin is required.

Crystalline Trypsin Solution & Soybean Trypsin Inhibitor Solution

Product Name	Catalogue No.	Unit Size	Storage Temp.
Crystalline Trypsin Solution (0.02%) Without Phenol Red	03-047-1A	500ml	-20°C
	03-047-1B	100ml	-20°C
Soybean Trypsin Inhibitor 50X Conc., 5mg/ml	03-048-1C	20ml	-20°C

Crude trypsin is often the subculturing agent of choice for cell dissociation/disaggregation of adherent cells, although the treatment may be cytotoxic if prolonged. Over-trypsinization is a common cause of subculture problems. Regarding the use of crude trypsin, some important facts must be noted:

- Cells must **NEVER** remain in the crude trypsin for longer than 3-5 minutes as they may be seriously damaged in the process (i.e. damage to the intracellular proteins).
- Cells should **NEVER** be left without a fluid layer.

The use of crystalline trypsin, rather than crude trypsin, most often performs better long-term cell growth in serum-free medium formulations. It is specifically formulated to have a gentle nature with much better cell viability, in which the cells are not subject to the vagaries of time and circumstance as when the cruder forms of trypsin are utilized.

Some of the advantages of crystalline trypsin versus the cruder trypsin forms:

1. Crystalline trypsin does not damage cells after prolonged exposure.
2. Crystalline trypsin does not require multiple-change procedures and thus is less labor-intensive.
3. Crystalline trypsin maintains better cell viability and enhances the process of cell passaging.
4. Crystalline trypsin is not as cytotoxic to cells with all the negative ramifications of crude trypsin.
5. Biological Industries' Crystalline Trypsin Solution also contains additives that protect the cell wall, enhancing cell viability.

In a serum-free culture environment, the cells must be separated by rapid centrifugation or by utilizing trypsin inhibitors such as Soybean Trypsin Inhibitor (SBTI). SBTI is a single polypeptide that forms a stable, stoichiometric, enzymically inactive complex with trypsin, thereby reducing the availability of trypsin by somewhat binding chymotrypsin. With Biological Industries' Soybean Trypsin Inhibitor Solution, any excess Crystalline Trypsin Solution may be completely neutralized, thereby avoiding the use of serum for this purpose. The cells may then be re-suspended successfully in a suitable growth medium.

The use of animal-derived components in Biopharmaceutical Manufacturing is experiencing ever-increasing regulatory scrutiny. Therefore, there is the need to develop non-animal source products for cell culture. Trypsin is an essential product for cell culture manipulation. However, it is purified from animal-source materials with one unfortunate notable disadvantage: contamination from variegated sources such as viruses, other potential adventitious agents and other unwanted enzymes.

Ordering Information

Product Name	Catalogue No.	Unit Size	Storage Temp.
Vero Cells			
NutriVero™ VP1, Animal Component-Free Serum-Free Medium for the Monolayer Culture of Vero Cells (NutriVero™ VP1, ACF SFM)	05-066-1A	500ml	2-8°C
	05-066-1B	100ml	2-8°C
NutriVero™ VP2, Animal Component-Free Serum-Free Medium for the Microcarrier Suspension Culture of Vero Cells (NutriVero™ VP2, ACF SFM)	05-067-1A	500ml	2-8°C
	05-067-1B	100ml	2-8°C
Hybridoma Cells			
DCCM-1 without L-Glutamine	05-010-1A	500ml	2-8°C
	05-010-1B	100ml	2-8°C
DCCM-1 10X Conc., Without L-Glutamine without Sodium Bicarbonate	05-010-5B	100ml	2-8°C
DCCM-2, without L-Glutamine	05-015-1A	500ml	2-8°C
	05-015-1B	100ml	2-8°C
DCCM-2 10X Conc., Without L-Glutamine, Without Sodium Bicarbonate	05-015-5A	500ml	2-8°C
	05-015-5B	100ml	2-8°C
Low Protein Media BSA-Free (LPM) Without L-Glutamine	05-040-1A	500ml	2-8°C
	05-040-1B	100ml	2-8°C
Low Protein Media BSA-Free (LPM) 10X Conc., Without L-Glutamine, Without Sodium Bicarbonate	05-040-5B	100ml	2-8°C
BIOGRO-1 Serum-Free Media Supplement 50X Conc.	05-600-1B	100ml	-20°C
	05-600-1C	20ml	-20°C
	05-600-1D	10ml	-20°C
	05-600-1T	2ml	-20°C
BIOGRO-2 Serum-Free Media Supplement 50X Conc.	05-610-1B	100ml	-20°C
	05-610-1C	20ml	-20°C
	05-610-1D	10ml	-20°C
	05-610-1T	2ml	-20°C
Anchorage-dependant Cells			
BIO-MPM-1, Multi-Purpose SFM Without L-Glutamine	05-060-1A	500ml	2-8°C
	05-060-1B	100ml	2-8°C

Product Name	Catalogue No.	Unit Size	Storage Temp.
CHO Cells			
BIOCHO-1 SFM Base Without L-Glutamine	05-061-1A	500ml	2-8°C
	05-061-1B	100ml	2-8°C
BIOGRO-CHO Serum-Free Media Supplement 100X Conc.	05-620-1E	50ml	-20°C
	05-620-1F	1ml	-20°C
	05-620-1H	5ml	-20°C
Mononuclear Cells (Immunology)			
BIOTARGET™-1 Without L-Glutamine	05-080-1A	500ml	2-8°C
	05-080-1B	100ml	2-8°C
Insect Cells			
BIOINSECT-1, With L-Glutamine	05-050-1A	500ml	2-8°C
Cryopreservation			
Serum-Free Cell Freezing Medium	05-065-1A	500ml	2-8°C
	05-065-1C	20ml	2-8°C
Auxiliary Solutions			
Crystalline Trypsin Solution (0.02%) Without Phenol Red	03-047-1A	500ml	-20°C
	03-047-1B	100ml	-20°C
Soybean Trypsin Inhibitor 50X Conc., 5mg/ml	03-048-1C	20ml	-20°C
Cell Dissociation Solution (non-enzymatic)	03-071-1B	100ml	2-8°C
Papain Dissociation Solution	03-072-1B	100ml	-20°C
Bovine Fibronectin Solution, 1mg/ml	03-090-1-01	1ml	2-8°C
	03-090-1-05	5ml	2-8°C
Human Fibronectin Solution, 1mg/ml	05-750-1H	1ml	2-8°C
	05-750-1F	5ml	2-8°C

Human Cytogenetics and Reproductive

Human Cytogenetics and Reproductive

3

Prenatal Diagnostics
Peripheral Blood
Bone Marrow
Karyotyping Reagents
Reproductive

3 Human Cytogenetics and Reproductive

Prenatal Diagnostics

Optimized Media for Culture and Genetic Analysis of Human Amniotic Fluid Cells and Chorionic Villi (CV) Samples

Chromosome Karyotyping was first developed in the field of Cytogenetics. The basic principle of the method is the preparation of chromosomes for microscopic observation by arresting cell mitosis at metaphase with colchicine and treating the cells with a hypotonic solution. This is followed by regular or fluorescent staining of the chromosomes, which are then tested with the aid of a microscope and computer programs to arrange and identify the chromosomes for the presence of genetic abnormalities.

In principle, this method enables the identification of any abnormality - excess chromosomes or chromosome deficiency, broken chromosomes, or excess genetic material (as a result of a recombination process). Clinical cytogenetics laboratories use this method with amniotic fluid, chorionic villi, blood cells, skin cells, and so on, which can be cell cultured to obtain mitotic cells.

Most amniotic fluid cells originate from the fetus and include fibroblasts, epithelial cells and amniocytes. The cells suited for genetic analysis are fibroblasts and amniocytes, and chromosome preparation from these cells yields a clear picture of the chromosomes for microscopic observation.

Amniocentesis is typically carried out in week 16-20 of pregnancy, when 20-40ml of amniotic fluid is drawn for genetic analysis.

The cells can be seeded on a slide or in suitable flasks to obtain colonies or cell cultures. Since the cells divide, chromosome karyotyping can be carried out on them for general genetic testing. To test specific abnormalities, a small number of cells can be taken from the original sample for FISH and/or QF-PCR testing.

The time that elapses until the final results of genetic analysis are obtained is of significant importance both from an emotional point of view - the tension and stress entailed in waiting for the final results - and a practical one - the need to terminate pregnancy if genetic abnormalities are found. Pregnancy termination in the second trimester in effect means performing an abortion; hence the importance of obtaining results as early as possible in order to alleviate the procedure.

In the past decade, Biological Industries Ltd. has developed a range of cytogenetics products, including media for culture of amniotic fluid and chorionic villi cells, BIOAMF™-1, BIOAMF™-2 and BIOAMF™-3, which are selling very successfully throughout the world.

BIOAMF™-1 Basal Medium and Supplement

Product Name	Catalogue No.	Unit Size
BIOAMF™-1 Basal Medium	01-190-1A	450ml
	01-190-1B	90ml
BIOAMF™-1 Supplement	01-192-1D	10ml
	01-192-1E	50ml

BIOAMF™-1 is designed for the primary culture of human amniotic fluid cells and chorionic villi (CV) samples in both open (5% CO₂) and closed systems.

The medium allows rapid growth of amniocytes or chorionic villi for use in karyotyping.

The medium consists of two components: basal medium and frozen supplement.

Instructions for Use

For the preparation of 500ml complete medium, use 01-190-1A with 01-192-1E. For the preparation of 100ml complete medium, use 01-190-1B with 01-192-1D. Thaw the BIOAMF™-1 Supplement by swirling in a 37°C water bath, and transfer the contents to the bottle of BIOAMF™-1 Basal Medium. Mix the complete medium by swirling the bottle, and add 2mM L-Glutamine (L-Glutamine Solution 200mM, cat. no. 03-020-1).

Antibiotics may be added if desired (Gentamicin, cat. no. 03-035-1).

Storage and Stability

BIOAMF™-1 Basal Medium is stable for 15 months from production date when stored at 2-8°C.

BIOAMF™-1 Supplement is stable for 24 months from production date when stored at -20°C.

The complete medium is stable for 14 days when stored at 2-8°C.

Do not freeze the complete medium. Protect both the basal medium and the complete medium from light.

BIOAMF™-2 Complete Medium

For faster results

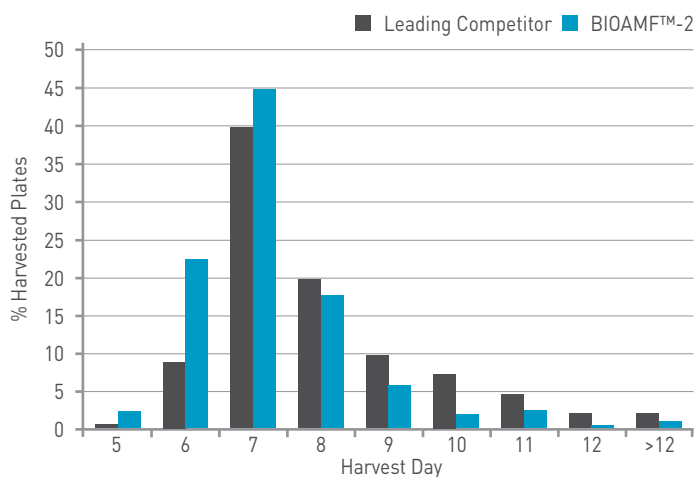
Product Name	Catalogue No.	Unit Size
BIOAMF™-2 Complete Medium	01-194-1A	500ml
	01-194-1B	100ml

BIOAMF™-2 is a complete medium specifically optimized for the primary culture of human amniotic fluid cells and chorionic villi (CV) samples in both open (5% CO₂) and closed systems.

No addition of serum is required, and chromosome karyotyping time is greatly reduced compared with the conventional medium.

Note: this is a one-bottle formulation, which also contains L-Glutamine and antibiotics. Simply thaw and use!

Figure 1: Comparison of the Percentage of Harvested Plates According To Harvest Day Between BIOAMF™-2 By Biological Industries and A Leading Competitor



Storage and Stability

BIOAMF™-2 Medium should be kept frozen at -20°C. After thawing, the medium should be stored at 2-8°C. The medium should be used within 7 days after thawing. Protect the medium from light.

Instructions for Use

Thaw BIOAMF™-2 Medium at refrigerator temperatures (2-8°C). Mix gently after thawing.

BIOAMF™-3 Complete Medium

For increased metaphase yield

Product Name	Catalogue No.	Unit Size
BIOAMF™-3 Complete Medium	01-196-1A	500ml
	01-196-1B	100ml

An improved version of complete medium specifically optimized for the primary culture of human amniotic fluid cells and chorionic villi samples used in prenatal diagnostic testing. This medium accelerates the growth of the non-epithelial cells used for chromosome karyotyping. The medium is supplied frozen and contains L-Glutamine and antibiotics.

Storage and Stability

BIOAMF™-3 Medium should be kept frozen at -20°C. After thawing, the medium should be stored at 2-8°C. The medium should be used within 14 days after thawing. Protect the medium from light.

Instructions for Use

Thaw BIOAMF™-3 Medium at refrigerator temperatures (2-8°C). Mix gently after thawing.

Peripheral Blood

Blood cell karyotyping is an important tool in modern human cytogenetics, providing information about chromosomal abnormalities, their frequency in the population, and the relationship between specific chromosomal abnormalities and phenotypic effects.

Human cytogenetic studies involve the examination of a stimulated lymphocyte after blocking cell division at metaphase with an inhibitor of spindle formation. The nuclear membrane breaks down and chromosome condensation takes place as usual, but the chromosomes fail to organize themselves into a metaphase plate.

This gives an appearance quite unlike a natural metaphase, in that the chromosomes are free within the cytoplasm. Subsequent processing and staining allows clear visualization of the chromosomes.

The chromosomes can be stained either by a technique that gives a fairly uniform intensity, or by a technique that gives differential staining along the length of the chromosome.

Advantages

- Saves time.
- Excellent growth promotion.
- No other supplements required.

Peripheral Blood Karyotyping Medium

Product Name	Catalogue No.	Unit Size
Peripheral Blood Karyotyping Medium Without Phytohemagglutinin	01-198-1A	500ml
	01-198-1B	100ml
Peripheral Blood Karyotyping Medium With Phytohemagglutinin	01-201-1A	500ml
	01-201-1B	100ml
	01-201-1H	5ml

Peripheral Blood (PB) Karyotyping Medium is specifically optimized for short-term culture of peripheral blood lymphocytes for chromosome analysis. No addition of serum, glutamine or antibiotics is required. The medium is supplied frozen.

Storage and Stability

PB Karyotyping Medium should be kept frozen at -20°C . After thawing, the medium should be stored at $2-8^{\circ}\text{C}$. The medium should be used within 10 days after thawing. Protect the medium from light.

Bone Marrow

Cytogenetic analysis of human hematopoietic cells using bone marrow aspirates is a standard practice in hematology. Cell culture improvements and processing techniques have enabled the identification of a number of recurring abnormalities in solid tumors and hematologic malignant diseases. But even more data are available for leukemias and lymphomas than for solid tumors because of the relative ease of obtaining bone marrow or peripheral blood specimens from leukemia patients.

The study of chromosomal abnormalities in leukemia serves two functions:

The first is to assist in more accurate diagnosis, thereby providing prognostic information and allowing the more rational selection of therapy for a particular patient. The second is to identify the sites of consistent rearrangements, providing the precise localization required for the isolation and cloning of DNA from these regions. Using molecular techniques the function of the genes can be identified and the mechanisms whereby their altered function is involved in tumorigenesis can be determined.

In the past, it was assumed that cytogenetic analysis of hematologic malignant disorders was best performed directly on uncultured bone marrow samples. However, later studies indicate that analysis of cultured samples disclosed a clonal abnormality that would not have been detected if the direct method alone had been used. Thus, for many samples, chromosomal rearrangements were often characterized only after analysis of cultured preparations.

Bone Marrow Karyotyping Medium

Product Name	Catalogue No.	Unit Size
Bone Marrow Karyotyping Medium Without conditioned medium	01-199-1A	500ml
	01-199-1B	100ml

Bone Marrow Karyotyping Medium is intended for use in short-term cultivation of primary bone marrow cells for chromosome evaluation. Bone Marrow Karyotyping Medium is based on RPMI-1640 basal medium supplemented with L-Glutamine, foetal bovine serum, and antibiotics (Gentamicin). The medium does not contain any mitogens or conditioned medium.

Bone Marrow Karyotyping Medium is supplied as frozen medium, which is ready for use after thawing.

Instructions for use

The bone marrow karyotyping method was developed to provide information about chromosomal abnormalities. The ready-to-use medium is intended for the culture of bone marrow cells without any mitogens or conditioned medium. After 48-72 hours, a mitotic inhibitor is added to the culture to stop mitosis in the metaphase stage.

After treatment by hypotonic solution, fixation and staining, chromosomes can be microscopically observed and evaluated for abnormalities.

Storage and Stability

Bone Marrow Karyotyping Medium should be kept frozen at -20°C. After thawing, the medium should be stored at 2-8°C. The medium should be used within 10 days after thawing. Protect the medium from light.

Bone Marrow Karyotyping Medium with Conditioned Medium

Product Name	Catalogue No.	Unit Size
Hematopoietic Cell Karyotyping Medium	01-200-1A	500ml
With conditioned medium	01-200-1B	100ml

Cytogenetic analysis of human hematopoietic cells using bone marrow aspirates is a standard practice in hematology. Fresh cells or cells grown in short-term cultures often yield an insufficient number of mitotic cells and repeated aspirations are required. Hematopoietic Cell Karyotyping Medium was developed to stimulate the proliferation of human hematopoietic cells from bone marrow as well as peripheral blood. This medium is particularly effective for karyotyping of acute non-lymphocytic leukemias and various stages of chronic myelogenous leukemia, as well as other hematological disorders such as myelodysplastic syndrome and polycythemia vera. Hematopoietic Cell Karyotyping Medium is based on MEM-Alpha basal medium supplemented with L-Glutamine, foetal bovine serum, antibiotics (Gentamicin) and conditioned medium.

Hematopoietic Cell Karyotyping Medium is supplied as frozen medium, which is ready for use after thawing.

Instructions for use

The hematopoietic cell karyotyping method was developed to provide information about chromosomal abnormalities. In the presence of a conditioned medium, acute and chronic nonlymphocytic leukemic cells in bone marrow and peripheral blood cultures are stimulated to enter into mitosis by DNA replication. After 48-72 hours, a mitotic inhibitor is added to the culture to stop mitosis in the metaphase stage.

After treatment by hypotonic solution, fixation and staining, chromosomes can be microscopically observed and evaluated for abnormalities.

Storage and Stability

Hematopoietic Cell Karyotyping Medium should be kept frozen at -20°C. After thawing, the medium should be stored at 2-8°C. The medium should be used within 10 days after thawing. Protect the medium from light.

Karyotyping Reagents

Phytohaemagglutinin M (PHA-M)

Product Name	Catalogue No.	Unit Size
Phytohaemagglutinin M (PHA-M), Lyophilized	12-006-1H	5ml
Phytohaemagglutinin M (PHA-M), Liquid	12-009-1H	5ml

Phytohaemagglutinin is a lectin extracted from red kidney beans (*Phaseolus vulgaris*). The protein consists of two molecular species, a leucoagglutinin (PHA-L) and an erythroagglutinin (PHA-E). Each of the proteins contains a family of five isolectins, each being a tetramer held together by noncovalent forces. PHA-M is the mucoprotein form and is a crude extract used for the stimulation of cell proliferation in lymphocyte culture. PHA-M also has a powerful erythroagglutinating property and it was originally used for separating leukocytes from whole blood. PHA-M from Biological Industries is sterile. Each lot is tested and standardized for mitotic stimulation using primary human peripheral blood lymphocytes.

Colchicine Solution, 10µg/ml in DPBS

Product Name	Catalogue No.	Unit Size
Colchicine Solution, 10µg/ml in DPBS	12-003-1C	25ml

Colcemid (Demecolcine) Solution, 10µg/ml in DPBS

Product Name	Catalogue No.	Unit Size
Colcemid Solution, 10µg/ml in DPBS	12-004-1D	10ml

Colcemid, N-deacetyl-N-methylcolchicine, is related to colchicine, but animal studies found it to be much less toxic. Colcemid arrests mitotic cultured cells in metaphase and it should be treated with care, since it is mutagenic, tumorigenic, and teratogenic.

Colcemid Solution from Biological Industries is prepared in PBS and it is recommended to use a concentration of 0.1µg/ml in culture medium. Colcemid is recommended for use in chromosome analysis during lymphocyte karyotyping and amniotic fluid cell chromosome analysis, and in cell synchronization.

Storage

Colcemid Solution should be stored at 2-8°C, protected from light.

Hypotonic solutions

A major step in harvesting cells for chromosome karyotyping is treatment with a hypotonic saline solution to increase cell volume. Hypotonic solutions work by creating a concentration gradient across the cytoplasmic membrane and water then rushes in by active transport.

Potassium Chloride 0.075 Molar

Product Name	Catalogue No.	Unit Size
Potassium Chloride 0.075 Molar	12-005-1B	100ml

Sodium Citrate Solution (0.8%)

Product Name	Catalogue No.	Unit Size
Sodium Citrate Solution (0.8%)	01-934-1A	500ml

Trypsin EDTA (0.5%), EDTA 0.2% , 10X Conc.

Product Name	Catalogue No.	Unit Size	Formulation Page
Trypsin-EDTA 10X	03-051-5B	100ml	
	03-051-5C	20ml	

Giemsa banding has become the most widely used technique for the routine staining of chromosomes. The most commonly used method to obtain this staining is to treat slides with trypsin. This procedure allows for chromosome digestion and high resolution staining.

Trypsin-EDTA 10X from Biological Industries contains Trypsin (1:250) 5gr per liter, and EDTA 2gr per liter, and it should be stored at -20°C.

Cell Synchronization Kit

Product Name	Catalogue No.	Unit Size
Cell Synchronization Kit	12-008-60	60 reactions

For high-resolution cytogenetic analysis

The blood cell karyotyping method was developed to provide information about chromosomal abnormalities. Lymphocyte cells do not normally undergo subsequent cell divisions. In the presence of a mitogen, lymphocytes are stimulated to enter into mitosis by DNA replication. After 48-72 hours, a mitotic inhibitor is added to the culture to stop mitosis in the metaphase stage. After treatment by hypotonic solution, fixation and staining, chromosomes can be microscopically observed and evaluated for abnormalities.

High resolution analysis is a special manipulation of the routine blood karyotyping procedure designed to provide a large number of mitotic figures in late prophase or prometaphase. At this stage of mitosis the chromosomes are longer and less condensed. After G-banding, the chromosomes will show greater level of band resolution not seen in routine analysis. High resolution allows more detailed analysis of the karyotype.

Cultures can be synchronized by the addition of methotrexate (MTX), an inhibitor of thymidine biosynthesis which blocks cells in the S-phase (DNA synthesis) of the cell cycle. After 16-18 hours, most of the dividing cells in the culture are in the S-phase. If thymidine is added to the culture, the MTX block is released and the cells proceed synchronously to mitosis, at which point colcemid may be added. A very short colcemid treatment in conjunction with this technique may be used to produce extended prometaphase chromosomes when small deletions or rearrangements are suspected.

Materials

1. Methotrexate (Amethopterin), 10-5M in HBSS: 4 vials containing 1.5ml each
2. Thymidine, 10-3M in distilled water: 4 vials containing 1.5ml each

Storage and Stability

The solutions must be kept frozen and protected from light. If appropriately stored, the solutions are stable for at least 18 months from the date of preparation.

Ordering Information

Product Name	Catalogue No.	Unit Size	Storage Temp.
Prenatal Diagnostics			
BIOAMF™-1 Basal Medium	01-190-1A	450ml	2-8°C
	01-190-1B	90ml	2-8°C
BIOAMF™-1 Supplement	01-192-1D	10ml	-20°C
	01-192-1E	50ml	-20°C
BIOAMF™-2 Complete Medium	01-194-1A	500ml	-20°C
	01-194-1B	100ml	-20°C
BIOAMF™-3 Complete Medium	01-196-1A	500ml	-20°C
	01-196-1B	100ml	-20°C
Peripheral Blood			
Peripheral Blood Karyotyping Medium Without Phytohemagglutinin	01-198-1A	500ml	-20°C
	01-198-1B	100ml	-20°C
Peripheral Blood Karyotyping Medium With Phytohemagglutinin	01-201-1A	500ml	-20°C
	01-201-1B	100ml	-20°C
	01-201-1H	5ml	-20°C
Bone Marrow			
Bone Marrow Karyotyping Medium Without conditioned medium	01-199-1A	500ml	-20°C
	01-199-1B	100ml	-20°C
Hematopoietic Cell Karyotyping Medium With conditioned medium	01-200-1A	500ml	-20°C
	01-200-1B	100ml	-20°C
Karyotyping Reagents			
Colchicine Solution, 10µg/ml in DPBS	12-003-1C	25ml	2-8°C
Colcemid Solution, 10µg/ml in DPBS	12-004-1D	10ml	2-8°C
Potassium Chloride, 0.075 Molar	12-005-1B	100ml	2-8°C
Phytohemagglutinin-M (PHA-M), Lyophilized	12-006-1H	5ml	2-8°C
Cell Synchronization Kit	12-008-60	60 reactions	-20°C
Phytohemagglutinin-M (PHA-M), Liquid	12-009-1H	5ml	-20°C
Sodium Citrate Solution (0.8%)	01-934-1A	500ml	AMB
Trypsin EDTA (0.5%), EDTA 0.2% 10X Conc ¹	03-051-5B	100ml	-20°C
	03-051-5C	20ml	-20°C

⁽¹⁾ See formulation on page 106

Reproductive

Modified Ham's F-10 with Gentamicin

Product Name	Catalogue No.	Unit Size
Modified Ham's F-10 With Gentamicin	01-925-1B	100ml

Designed for in-vitro sperm washing procedures which do not require CO₂ incubation. Modified Ham's F-10 contains HEPES and Sodium Bicarbonate.

This buffering system provides optimum pH maintenance (7.2-7.4) and does not require the use of a CO₂ incubator.

Contains

Ham's F-10 supplemented with:

- 21mM HEPES.
- 4mM Sodium Bicarbonate.
- Human Serum Albumin (for human use, USP).
- Sodium Lactate.
- Gentamicin.
- Phenol Red.

Protein supplementation

The medium contains 0.6% Human Serum Albumin (HSA). The HSA used in the manufacture of this medium is approved for human use.

Storage

Store at 2-8°C.

Quick Stain

Product Name	Catalogue No.	Unit Size
Quick Stain	01-939-1U	3ml for 200-300 slides

A dye mixture for staining and visualization of semen specimens.

Quick Stain is an in-vitro diagnostic rapid test for spermatozoa staining and morphology assessment. It also permits classification of round cells in semen -mainly immature germ cells and leukocytes. Excessive numbers of leukocytes may be associated with infection and poor sperm quality. With Quick Stain, nuclei are stained dark-purple while acrosome, tail and other cell structures have different shades of violet.

Storage

The stain solution bottle should be kept tightly closed.

Quick Stain should be protected from light and stored at 2-8°C.

Classical Media and Reagents

Classical Media and Reagents

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- Single Strength Liquid Media
- Two Fold Concentration Media
- Five & Ten Fold Concentration Media
- Media for Insect Cells
- Powdered Media
- Reagents & Supplements
- Biochemicals
- Balanced Salt Solutions

4 Classical Media and Reagents

4

Classical Media and Reagents

Sterile Filtration and Aseptic Filling

Biological Industries' medium products are prepared by a sterile filtration process and aseptic filling. The process has been validated to ensure that the production of solutions meets the sterility assurance level of 10^3 .

The filtration step includes the use of sterilizing grade membrane filters. After filtration, the medium is dispensed into bottles by an aseptic filling process which has been validated to insure sterility of the final product. Medium products are produced in a controlled environment (clean rooms) designed to carefully control air pressure and particulate matter.

The manufacturing area is a class 100,000 (ISO 8) environment. The sterile bottles and equipment are stored in a class 10,000 (ISO 7) environment, and the filling room is a class 1000 (ISO 6) environment with class 100 (ISO 5) laminar air flow sterile bench.

Clean rooms are monitored on a regular basis for particulate and microbial levels to ensure that the air handling system, cleaning protocols and personnel maintain required standards. After filling, the final product is held in quarantine until all quality control tests have been completed.

Quality Control

The quality of our liquid media is confirmed by testing representative samples from each lot.

Physicals Tests

pH & osmolality are measured to verify compliance with accepted specifications.

Endotoxins

Endotoxin concentrations are routinely measured with the Limulus Amebocyte Lysate (LAL) test using the kinetic turbidimetric method.

Sterility Testing

The absence of fungal and bacterial contamination is confirmed by sterility tests using the direct inoculation method or membrane filtration method with microbiological media. All media containing products of animal origin are tested for the absence of mycoplasma.

Cell Growth Promotion

The growth promotion activity and the absence of cytotoxicity of all medium products are tested using appropriate cell lines. Cells are examined for doubling time and cell morphology.

Expiration Date

Refer to product label for expiration date.

Storage

For optimal performance, store medium products under the conditions specified on the label.

Avoid light exposure of liquid medium products.

Certificate of Analysis and Safety Data

A Certificate of Analysis for each product lot is available upon request as well as a Material Safety Data Sheet (MSDS).

Single-Strength Liquid Media

Product Name	Catalogue No.	Unit Size	Storage Temp.	Formulation Page
Basal Medium-Eagle (BME) Earle's Salts Base Without L-Glutamine	01-015-1A	500ml	2-8°C	95
Minimum Essential Medium-Eagle (MEM-E) Earle's Salts Base Without L-Glutamine	01-025-1A 01-025-1B	500ml 100ml	2-8°C 2-8°C	99 99
Minimum Essential Medium-Eagle (MEM-H) Hanks' Salts Base Without L-Glutamine	01-035-1A	500ml	2-8°C	99
Minimum Essential Medium-Eagle (MEM-NEAA) Earle's Salts Base With Non-Essential Amino Acids Without L-Glutamine	01-040-1A	500ml	2-8°C	99
Minimum Essential Medium-Alpha (MEM-A) With 1g/l D-Glucose (Low Glucose) With L-Glutamine Without Ribonucleosides and Deoxyribonucleosides	01-042-1A	500ml	2-8°C	96
Minimum Essential Medium Alpha (MEM-A) With 4.5 g/l D-Glucose (High Glucose) With L-Glutamine Without Ribonucleosides and Deoxyribonucleosides	01-043-1A	500ml	2-8°C	
Minimum Essential Medium (MEM) for suspension cultures Without L-Glutamine	01-045-1A	500ml	2-8°C	99

Product Name	Catalogue No.	Unit Size	Storage Temp.	Formulation Page
Dulbecco's Modified Eagle Medium (DMEM) With 1g/l D-Glucose (Low Glucose) With Sodium Pyruvate 110mg/l Without L-Glutamine	01-050-1A 01-050-1B	500ml 100ml	2-8°C 2-8°C	95-96 95-96
Dulbecco's Modified Eagle Medium (DMEM) With 4.5g/l D-Glucose (High Glucose) Without Sodium Pyruvate Without Phenol Red Without L-Glutamine	01-053-1A	500ml	2-8°C	
Dulbecco's Modified Eagle Medium (DMEM) With 4.5g/l D-Glucose (High Glucose) Without Sodium Pyruvate Without L-Methionine Without L-Glutamine	01-054-1A	500ml	2-8°C	
Dulbecco's Modified Eagle Medium (DMEM) With 4.5g/l D-Glucose (High Glucose) Without Sodium Pyruvate Without L-Glutamine	01-055-1A	500ml	2-8°C	95-96
Dulbecco's Modified Eagle Medium (DMEM) With 4.5g/l Glucose (High Glucose) Without Sodium Pyruvate With stable Glutamine	01-056-1A	500ml	2-8°C	
Dulbecco's Modified Eagle Medium (DMEM) Without D-Glucose Without Sodium Pyruvate Without L-Glutamine	01-057-1A	500ml	2-8°C	
Iscove's Modified Dulbecco's Medium (IMDM) With L-Glutamine Without Alpha-Thioglycerol Without Beta Mercaptoethanol	01-058-1A	500ml	2-8°C	95-96
MCDB-153 (Modified)	01-059-1A	500ml	2-8°C	
McCoy's 5A Medium (Modified) Without Serum With L-Glutamine	01-075-1A 01-075-1B	500ml 100ml	2-8°C 2-8°C	97 97
Medium M-199 (M199E) Earle's Salts Base With L-Glutamine	01-080-1A 01-080-1B	500ml 100ml	2-8°C 2-8°C	98 98

Product Name	Catalogue No.	Unit Size	Storage Temp.	Formulation Page
Medium M-199 (M199H) Hanks' Salts Base With L-Glutamine	01-085-1A 01-085-1B	500ml 100ml	2-8°C 2-8°C	98 98
Nutrient Mixture F-10 (Ham's) With L-Glutamine	01-090-1A 01-090-1B	500ml 100ml	2-8°C 2-8°C	100 100
Nutrient Mixture F-12 (Ham's) With L-Glutamine	01-095-1A	500ml	2-8°C	100
RPMI Medium 1640 With L-Glutamine	01-100-1A 01-100-1B	500ml 100ml	2-8°C 2-8°C	97 97
RPMI Medium 1640 Without D-Glucose Without L-Glutamine	01-101-1A 01-101-1B	500ml 100ml	2-8°C 2-8°C	
RPMI Medium 1640 Without Phenol Red Without L-Glutamine	01-103-1A 01-103-1B	500ml 100ml	2-8°C 2-8°C	
RPMI Medium 1640 Without L-Glutamine	01-104-1A 01-104-1B	500ml 100ml	2-8°C 2-8°C	
RPMI Medium 1640 With 25mM Hepes With L-Glutamine	01-106-1A 01-106-1B	500ml 100ml	2-8°C 2-8°C	97 97
Waymouth's MB 752/1 Medium With L-Glutamine	01-110-1A	500ml	2-8°C	101
Leibovitz L-15 Medium With L-Glutamine	01-115-1A	500ml	2-8°C	99
Dulbecco's Modified Eagle Medium (DMEM): Nutrient Mixture F-12 (Ham's) (1:1) Without L-Glutamine With Sodium Bicarbonate 1.2g/l With Hepes 15mM With Sodium Pyruvate 55mg/l	01-170-1A	500ml	2-8°C	100
Mouse Embryonic Stem Cells (ESC) Basal Medium With Stable Glutamine	01-171-1A	500ml	2-8°C	

Two Fold Concentration Media

Product Name	Catalogue No.	Unit Size	Storage Temp.	Formulation Page
Minimum Essential Medium-Eagle (MEM-E) 2X Conc. Earle's Salts Base Without L-Glutamine With Sodium Bicarbonate	01-025-9A	500ml	2-8°C	
Dulbecco Modified Eagle Medium (DMEM), 2X Conc. 4.5g/l D-Glucose (High Glucose) Without L-Glutamine With Sodium Bicarbonate	01-055-9A	500ml	2-8°C	

Five-Fold and Ten Fold Concentration Media

Product Name	Catalogue No.	Unit Size	Storage Temp.	Formulation Page
Basal Medium Eagle (BME) 10X Conc. Earle's Salts Base Without L-Glutamine Without Sodium Bicarbonate	01-015-5A	500ml	2-8°C	95
Minimum Essential Medium Eagle (MEM-E) 10X Conc. Earle's Salts Base Without L-Glutamine Without Sodium Bicarbonate	01-025-5A	500ml	2-8°C	99
Dulbecco Modified Eagle Medium (DMEM) 5X Conc. 1g/l D-Glucose (Low Glucose) Without L-Glutamine Without Sodium Bicarbonate	01-050-4A	500ml	AMB	95-96
Dulbecco Modified Eagle Medium (DMEM) 5X Conc. 4.5g/l D-Glucose (High Glucose) Without L-Glutamine Without Sodium Bicarbonate	01-055-4A 01-055-4B	500ml 100ml	AMB AMB	95-96 95-96
Medium M-199 10X Conc. Earle's Salts Base With L-Glutamine Without Sodium Bicarbonate	01-080-5A	500ml	2-8°C	98
Nutrient Mixture F-10 (Ham's) 10X Conc. With L-Glutamine Without Sodium Bicarbonate	01-090-5A	500ml	2-8°C	
Nutrient Mixture F-12 (Ham's) 10X Conc. With L-Glutamine Without Sodium Bicarbonate	01-095-5A	500ml	2-8°C	
RPMI Medium 1640 10X Conc. Without L-Glutamine Without Sodium Bicarbonate	01-104-5A	500ml	2-8°C	97

Media Preparation**Directions for the Preparation of Single Strength Synthetic Liquid Media (1x) from Concentrated Media.**

1. Measure out sterile culture - grade water (Catalogue No. 03-055-1) to approximately 70% of desired total volume of media. Pour water into an appropriate sterile mixing container that is close to the desired final volume. The water should be at room temperature.
2. Add the amount of the concentrated medium or concentrated medium components.
3. Add the desired amount of L-Glutamine Solution 200 mM (Catalogue No. 03-020-1) if required.
4. Add the desired amount of Sodium Bicarbonate Solution 7.5% (Catalogue No. 03-040-1).
5. Add antibiotics solution if desired.
6. Add water to the final volume. During the dilution, stir gently into equilibrium. If necessary, adjust pH with sterile 1 N NaOH or HCl.
7. Add the desired amount of serum, if required.
8. Store at 2°C to 8°C.

Important

The above procedures are carried out under strict sterile conditions. Do not use mouth pipetting.

Example 1**Preparation of Basal Medium-Eagle, Earle's Salt Base, one liter**

1. 700 ml sterile water (Catalogue No.03-055-1).
2. 100 ml Basal Medium-Eagle, Earle's Salts Base, concentrate 10X, without Sodium Bicarbonate and L-Glutamine (Catalogue No. 01-015-5).
3. 10 ml L-Glutamine Solution 200 mM (Catalogue No. 03-020-1).
4. 29.4 ml Sodium Bicarbonate Solution 7.5%. (Catalogue No. 03-040-1).
5. 10ml Penicillin-Streptomycin Solution (Catalogue No. 03-031-1).
6. Sterile water to final volume, Adjust pH if necessary.

Example 2**Preparation of RPMI from Concentrate**

Our RPMI Concentrate is prepared by a special method which enhances the stability of the product. Therefore in this case proceed as follows:

1. 700 ml distilled water.
2. Add 100 ml RPMI Concentrate 10X.
3. Adjust pH to 6.5-7.0 with 1N NaOH.
4. Add 10.3 ml L-Glutamine Solution 200 mM.
5. Add 26.7 ml Sodium Bicarbonate Solution 7.5%.
6. Adjust pH with 1N NaOH or 1N HCl to pH 7.0-7.4.
7. Add distilled water to final volume. Adjust pH if necessary.
8. Filter the medium into sterile containers using a 0.2 µm membrane filter.

Recommended Amounts of Sodium Bicarbonate and L-Glutamine To Be Added In The Preparation of Single Strength Liquid Media (1x) from Concentrated Media (5x, 10x)

Desired product Cat. No. / Description	Prepared From product Cat. No. / Description	Quantity Sodium Bicarbonate Solution 7.5% Cat. No. 03-040-1 ml/Liter	Quantity L-Glutamine Solution 200mM Cat. No. 03-020-1 ml/Liter
01-015-1 Basal Medium-Eagle Earle's Salts Base (1x)	01-015-5 Basal Medium-Eagle Earle's Salts Base (10x)	29.4	10
01-025-1 Minimum Essential Medium Eagle Earle's Salts Base (1x)	01-025-5 Minimum Essential Medium Eagle Earle's Salts Base (10x)	29.4	10
01-050-1 Dulbecco's Modified Eagle Medium Low Glucose (1x)	01-050-4 Dulbecco's Modified Eagle Medium Low Glucose (5x)	49.4	20
01-055-1 Dulbecco's Modified Eagle Medium High Glucose (1x)	01-055-4 Dulbecco's Modified Eagle Medium High Glucose (5x)	49.4	20
01-080-1 Medium M-199 Earle' Salt Base (1x)	01-080-5 Medium M-199 Earle' Salt Base (10x)	29.4	
01-085-1 Medium M-199 Hanks' Salt Base (1x)	01-085-5 Medium M-199 Hanks' Salt Base (10x)	4.7	
01-090-1 Nutrient Mixture F-10 (HAM) (1x)	01-090-5 Nutrient Mixture F-10 (HAM) (10x)	16	
01-095-1 Nutrient Mixture F-12 (HAM) (1x)	01-095-5 Nutrient Mixture F-12 (HAM) (10x)	15.7	
01-100-1 RPMI-1640 (1x)	01-104-5 RPMI-1640 (10x)	26.7	10.3
01-170-1 DMEM:F-12(1:1) (1x)	01-170-5 DMEM:F-12(1:1) (10x)	16	12.5

We recommend using cell culture grade water Cat. No. 03-055-1

L-Alanyl L-Glutamine Solution

Product Name	Catalogue No.	Unit Size	Storage Temp.
L-Alanyl-L-Glutamine (Stable Glutamine) 200 mM in 0.85% NaCl	03-022-1B	100ml	-20°C
	03-022-1C	20ml	-20°C

L-Alanyl L-Glutamine is a dipeptide substitute for L-Glutamine.

- Can be used as a direct substitute for L-Glutamine at equimolar concentrations in mammalian cell culture systems.
- Eliminates problems associated with the spontaneous breakdown of L-Glutamine during incubation.
- Highly soluble in aqueous solution and is heat stable.

Expiration

24 months

Storage

-20°C

Media for Insect Cells

Product Name	Catalogue No.	Unit Size	Storage Temp.	Formulation Page
BIOINSECT-1 Serum-Free medium With Glutamine	05-050-1A	500ml	2-8°C	
Schneider's <i>Drosophila</i> Medium With L-Glutamine	01-150-1A	500ml	2-8°C	101
Grace's Insect Cell Medium Without Insect Haemolymph Without Lactalbumin Hydrolysate Without Yeastolate With L-Glutamine	01-155-1A	500ml	2-8°C	101

Powdered Media

Product Name	Catalogue No.	Unit Size	Storage Temp.	Formulation Page
Minimum Essential Medium-Eagle (MEM-E) Powder Earle's Salts Base With L-Glutamine Without Sodium Bicarbonate	11-025-1N 11-025-1M 11-025-1G 11-025-1K	1x50 lt 1x10 lt 1x5 lt 1x1 lt	2-8°C 2-8°C 2-8°C 2-8°C	103 103 103 103
Minimum Essential Medium-Eagle (MEM-E) Powder, Earle's Salts Base With Non-Essential Amino Acids With L-Glutamine Without Sodium Bicarbonate	11-040-1N 11-040-1M 11-040-1G	1x50 lt 1x10 lt 1x5 lt	2-8°C 2-8°C 2-8°C	105 105 105
Minimum Essential Medium-Alpha (MEM-A) Powder With 1g/l D-Glucose (Low Glucose) Without Ribonucleosides and Deoxyribonucleosides With L-Glutamine Without Sodium Bicarbonate	11-042-1N 11-042-1M 11-042-1G 11-042-1K	1x50 lt 1x10 lt 1x5 lt 1x1 lt	2-8°C 2-8°C 2-8°C 2-8°C	103 103 103 103
Dulbecco's Modified Eagle Medium (DMEM) Powder With 1g/l D-Glucose (Low Glucose) With Sodium Pyruvate 110mg/l With L-Glutamine Without Sodium Bicarbonate	11-050-1N 11-050-1M 11-050-1G 11-050-1K	1x50 lt 1x10 lt 1x5 lt 1x1 lt	2-8°C 2-8°C 2-8°C 2-8°C	103 103 103 103
Dulbecco's Modified Eagle Medium (DMEM) Powder With 4.5g/l D-Glucose (High Glucose) Without Sodium Pyruvate With L-Glutamine Without Sodium Bicarbonate	11-055-1N 11-055-1M 11-055-1G 11-055-1K	1x50 lt 1x10 lt 1x5 lt 1x1 lt	2-8°C 2-8°C 2-8°C 2-8°C	103 103 103 103
Iscove's Modified Dulbecco Medium (IMDM) Powder With Hepes With L-Glutamine Without Sodium Bicarbonate	11-058-1N 11-058-1M 11-058-1G	1x50 lt 1x10 lt 1x5 lt	2-8°C 2-8°C 2-8°C	105 105 105
Medium M-199 (M199E) Powder Earle's Salts Base With L-Glutamine Without Sodium Bicarbonate	11-080-1N 11-080-1M 11-080-1G 11-080-1K	1x50 lt 1x10 lt 1x5 lt 1x1 lt	2-8°C 2-8°C 2-8°C 2-8°C	103-104 103-104 103-104 103-104
Nutrient Mixture F-10 (Ham's) Powder With L-Glutamine Without Sodium Bicarbonate	11-090-1N 11-090-1M 11-090-1G 11-090-1K	1x50 lt 1x10 lt 1x5 lt 1x1 lt	2-8°C 2-8°C 2-8°C 2-8°C	103-104 103-104 103-104 103-104

Product Name	Catalogue No.	Unit Size	Storage Temp.	Formulation Page
Nutrient Mixture F-12 (Ham's) Powder With L-Glutamine Without Sodium Bicarbonate	11-095-1N	1x50 lt	2-8°C	103-104
	11-095-1M	1x10 lt	2-8°C	103-104
	11-095-1G	1x5 lt	2-8°C	103-104
	11-095-1K	1x1 lt	2-8°C	103-104
RPMI Medium 1640, Powder With L-Glutamine Without Sodium Bicarbonate	11-100-1N	1x50 lt	2-8°C	105
	11-100-1M	1x10 lt	2-8°C	105
	11-100-1G	1x5 lt	2-8°C	105
	11-100-1K	1x1 lt	2-8°C	105
Dulbecco's Modified Eagle Medium (DMEM): Nutrient Mixture F-12 (Ham's) (1:1), Powder With Hepes 15mM With L-Glutamine Without Sodium Bicarbonate	11-170-1N	1x50 lt	2-8°C	103-104
	11-170-1M	1x10 lt	2-8°C	103-104
	11-170-1G	1x5 lt	2-8°C	103-104
	11-170-1K	1x1 lt	2-8°C	103-104
Dulbecco's Phosphate Buffered Saline (DPBS), Powder Without Calcium Chloride Without Magnesium Chloride With L-Glutamine Without Sodium Bicarbonate	11-223-1M	1x10 lt	2-8°C	
	11-223-1G	1x5 lt	2-8°C	
	11-223-1K	1x1 lt	2-8°C	

Unit Sizes: 1 liter, 5 liters, 10 liters and 50 liters.

Other products and package sizes are available by special order.

Powdered Media Preparation Procedure:

- To a mixing container that is as close to the final volume as possible, add 10% less distilled water than the desired total volume of medium.
- Add powdered medium to room temperature water with gentle stirring. Do not heat water.
- Rinse inside of package to remove all trace of powder.
- Add Sodium Bicarbonate as required.
- Dilute the medium to the desired volume with distilled water and stir until dissolved. Do not overmix.
- Adjust the pH to between 0.2-0.3 below the desired final working pH by slowly adding, with stirring, 1N NaOH HCl. The pH usually will rise 0.2 -0.3 units upon filtration. Keep the container closed until the medium is filtered.
- Process the medium immediately into sterile containers by membrane filtration using 0.2µ membrane filter.

See L-Alanyl L-Glutamine Solution on previous page.

Sodium Bicarbonate Concentrations:

Catalogue No.	Sodium Bicarbonate gram/liter	Sodium Bicarbonate ml/liter from 7.5% Solution
11-025-1	2.2	29.3
11-040-1	2.2	29.3
11-042-1	2.2	29.3
11-050-1	3.7	49.3
11-055-1	3.7	49.3
11-058-1	3.024	40.32
11-075-1	2.2	29.3
11-080-1	2.2	29.3
11-090-1	1.2	16
11-095-1	1.176	15.68
11-100-1	2.0	26.67
11-170-1	1.2	16

Example

RPMI 1640 (11-100-1) – 1 liter

- Prepare 900ml of distilled water in clean glass beaker. Water temperature should be 15-30°C. Put the beaker on a stirrer and add a stirring bar.
- Add the 1-liter powder to the water and stir gently. Fill some distilled water into the empty package, stir and pour the remains into the beaker. Stir until completely dissolved.
- Add 2.0 gram Sodium Bicarbonate (or 26.67 ml of 7.5% Sodium Bicarbonate Solution).
- Adjust pH to 0.1-0.3 units below the required pH using 1N HCl or 1N NaOH. The pH will rise by 0.1-0.3 units after filtration.
- Add distilled water up to 1 liter.
- Filter for sterility with 0.2µ membrane filter into sterile bottles.
- For the preparation of 10 liters multiply by 10.

Reagents and Supplements

Biological Industries' reagents and supplements are specifically designed for cell culture allowing for the growth and propagation of a wide spectrum of cell types under controlled conditions. In vitro cell culture systems provide the researcher with the appropriate means for effectively studying cell growth and differentiation, in order to understand the cellular response to specific environmental stimuli. Each and every cell culture system is designed to meet its nutritional and metabolic niche requirements once a basic medium is chosen. In order to realize maximum yields, the cell culture must have a large reserve of not only the basic nutrients, but also the essential requirements which provide an energy source, amino acids, vitamins and other various supplementations to enhance cell growth and performance. Biological Industries' reliable and proven products will help you reach your goals.

Biological Industries' products are not only well-known in many medical centers and hospitals nationally and internationally, but also in many prominent and renowned research laboratories in such diverse fields as cell culture and biology, immunology and oncology, virology, microbiology and parasitology, as well as in vitro fertilization among many other specialized fields. Customized solutions and media formulations are manufactured to exact specifications under strict and rigorous Quality Assurance/Quality Control Guidelines and supplied to numerous biotech and biopharmaceutical firms and corporations, from small-volume to large-scale operations, in order to meet your needs and requirements. All media are performance tested with applicable documentation that meets international standards, in order to ensure lot-to-lot uniformity and the highest quality. Bulk pricing is available upon request.

The following is a list of reagents and supplements that all undergo extensive and vigorous Quality Control Protocols.

Product Name	Conc.	Catalogue No.	Unit Size	Storage Temp.
BME Amino Acids Solution ⁽¹⁾ Without L-Glutamine	100X	01-315-1B	100ml	2-8°C
BME Vitamins Solution ⁽¹⁾	100X	01-316-1B	100ml	-20°C
MEM Amino Acids Solution Without L-Glutamine	50X	01-325-1B	100ml	2-8°C
MEM Vitamins Solution ⁽¹⁾	100X	01-326-1B	100ml	-20°C
MEM Non-Essential Amino Acids Solution ⁽¹⁾	100X	01-340-1B	100ml	2-8°C
Ribonucleosides and Deoxyribonucleosides for MEM-Alpha ⁽²⁾	500X	01-343-1D	10ml	-20°C
Lactalbumin Hydrolysate Solution, 166.6 gr/liter	50X	01-356-1B	100ml	2-8°C

Product Name	Conc.	Catalogue No.	Unit Size	Storage Temp.
Yeastolate Solution, 166.6 gr/liter	50X	01-357-1B	100ml	2-8°C
Human Recombinant Insulin Solution, (~3.5 mg/ml)	100 Units/ml	01-818-1H	5ml	2-8°C
Gelatin Solution	0.1%	01-944-1A 01-944-1B	500ml 100ml	2-8°C
Bovine Albumin Solution, Fraction V in saline	10%	03-010-1B	100ml	-20°C
Ethylenediaminetetraacetic Acid (EDTA) Disodium Salt Solution, in DPBS	0.05%	03-015-1B	100ml	AMB
L-Glutamine Solution, 29.2 mg/ml in Saline	200mM	03-020-1A 03-020-1B 03-020-1C	500ml 100ml 20ml	-20°C -20°C -20°C
L-Alanyl L-Glutamine (Stable Glutamine)	200 mM	03-022-1B 03-022-1C	100ml 20ml	-20°C -20°C
HEPES Buffer Solution, pH 7.3 at 37°C	1M	03-025-1B 03-025-1C	100ml 20ml	AMB AMB
Sodium Bicarbonate Solution	7.5%	03-040-1A 03-040-1B	500ml 100ml	AMB AMB
Sodium Bicarbonate Solution	5%	03-041-1A 03-041-1B	500ml 100ml	AMB AMB
Sodium Pyruvate Solution, 11.0 mg/ml	100mM	03-042-1B	100ml	-20°C
Water, Cell Culture Grade		03-055-1A	500ml	AMB
SPGA EDTA Solution		03-060-1A	500ml	-20°C
SPGA Solution		03-061-1A	500ml	-20°C
HAT Supplement, (Hypoxanthine 680.5 mg/l, Aminopterin 8.81 mg/l, Thymidine 193.8 mg/l), in DPBS	50X	03-080-1B	100ml	-20°C
HT Supplement (Hypoxanthine 680.5mg/l, Thymidine 193.8mg/l), in DPBS	50X	03-085-1B 03-085-1C	100ml 20ml	-20°C -20°C
Bovine Fibronectin Solution	1mg/ml	03-090-1-01 03-090-1-05	1ml 5ml	2-8°C 2-8°C
Phenol Red Solution, in DPBS	5mg/ml	03-100-1B	100ml	AMB
Trypan Blue Solution, in Saline	5mg/ml	03-102-1B	100ml	AMB
Human Fibronectin Solution	1mg/ml 1mg/ml	05-750-1H 05-750-1F	1ml 5ml	2-8°C 2-8°C

⁽¹⁾ See formulation on page 102

⁽²⁾ See formulation on page 96

Biochemicals

These cell culture-tested chemicals are regularly being used by Biological Industries for the manufacture of cell culture products and can be purchased separately.

Product Name	Catalogue No.	Unit Size
L-Alanine	41-239-25	25 gr
	41-239-100	100 gr
L-Arginine Free Base	41-219-25	25 gr
	41-219-100	100 gr
L-Arginine Hydrochloride	41-201-25	25 gr
	41-201-100	100 gr
L-Asparagine Monohydrate	41-215-25	25 gr
	41-215-100	100 gr
L-Aspartic Acid	41-216-25	25 gr
	41-216-100	100 gr
L-Cysteine Hydrochloride Hydrate	41-241-25	25 gr
	41-241-100	100 gr
L-Cystine	41-223-25	25 gr
	41-223-100	100 gr
L-Glutamic Acid	41-217-25	25 gr
	41-217-100	100 gr
L-Glutamine	41-218-25	25 gr
	41-218-100	100 gr
Glycine	41-202-25	25 gr
	41-202-100	100 gr
L-Histidine Hydrochloride Monohydrate	41-203-25	25 gr
	41-203-100	100 gr
L-Isoleucine	41-204-25	25 gr
	41-204-100	100 gr
L-Leucine	41-205-25	25 gr
	41-205-100	100 gr
L-Lysine Monohydrochloride	41-206-25	25 gr
	41-206-100	100 gr
L-Methionine	41-207-25	25 gr
	41-207-100	100 gr
L-Phenylalanine	41-208-25	25 gr
	41-208-100	100 gr
L-Proline	41-221-25	25 gr
	41-221-100	100 gr
L-Serine	41-209-25	25 gr
	41-209-100	100 gr
L-Threonine	41-210-25	25 gr
	41-210-100	100 gr

Product Name	Catalogue No.	Unit Size
L-Tryptophan	41-211-25	25 gr
	41-211-100	100 gr
L-Tyrosine	41-222-25	25 gr
	41-222-100	100 gr
L-Valine	41-212-25	25 gr
	41-212-100	100 gr
Bovine Serum Albumin Fraction V	41-903-25	25 gr
	41-903-100	100 gr
EDTA Disodium Dihydrate	41-922-25	25 gr
	41-922-100	100 gr
Gentamycin Sulfate	41-503-1	1 gr
	41-503-5	5 gr
Glucose Anhydrous	41-302-500	500 gr
Hepes	41-122-25	25 gr
	41-122-100	100 gr
Insulin, Human Recombinant	41-975-100	100 gr
Kanamycin Sulfate	41-507-1	1 gr
	41-507-5	5 gr
MOPS	41-811-50	50 gr
	41-811-100	100 gr
Neomycin Sulfate	41-505-1	1 gr
	41-505-5	5 gr
Nystatin	41-506-1	1 gr
	41-506-5	5 gr
Penicillin G Sodium	41-501-10	10 gr
	41-501-25	25 gr
	41-501-100	100 gr
Streptomycin Sulfate	41-502-25	25 gr
	41-502-100	100 gr
Collagen Type I, Rat Tail	01-990-100	100 gr
Transferrin, Human, Substantially Iron-Free	41-951-100	100 gr
Transferrin, Human, Iron-Saturated	41-951-500	500 gr
Trypsin, Porcine Pancreas (1:250)	41-920-25	25 gr
	41-920-100	100 gr

Balanced Salt Solutions

Balanced Salt Solutions, for all intents and purposes, are inorganic salt solutions that form the basis of many complex media formulations. They may contain varying amounts of NaCl, KCl, MgCl₂, NaHCO₃, MgSO₄, CaCl₂ and other salts, and have since been modified and enriched along with amino acids, vitamins, fatty acids and lipids and as well as other nutrients that segue into a final medium based upon application and technique to meet the cells unique niche requirements. These precise media formulations are now optimized to the nth degree to support a wide array of cell lines. The current role of a balanced salt solution in cell culture is multi-faceted and may be divided into four principal functions.

- Functions as a diluent, as an irrigating medium or transporting fluid while maintaining osmoregulation, the optimal and constant balance of osmotic pressure gradients between the intracellular and extracellular compartments.
- Provides cells with fluids and certain bulk inorganic ions essential for normal cell metabolism.
- When combined with a carbohydrate, such as Glucose (C₆H₁₂O₆), it provides a primary energy source for cell metabolism.
- When provided with a buffering system, it facilitates the maintenance of physiological pH within an acceptable range of 7.1-7.5.

Biological Industries offers a wide range of various formulations to meet all the requirements for cell culture. Each batch undergoes extensive and vigorous Quality Control Protocols to verify compliance with product specifications. Each batch undergoes a series of Chemical, Microbiological, Stability, and Performance Testing.

Product Name	Conc.	Catalogue No.	Unit Size	Storage Temp.
Gey's Balanced Salt Solution	1X	01-919-1A	500ml	AMB
Earle's Balanced Salt Solution	1X	02-010-1A 02-010-1B	500ml 100ml	AMB AMB
Earle's Balanced Salt Solution Without Sodium Bicarbonate	10X	02-010-5A 02-010-5B	500ml 100ml	AMB AMB
Earle's Balanced Salt Solution Without Phenol Red	1X	02-011-1A 02-011-1B	500ml 100ml	AMB AMB
Earle's Balanced Salt Solution Without Phenol Red Without Sodium Bicarbonate	10X	02-011-5A 02-011-5B	500ml 100ml	AMB AMB
Hanks' Balanced Salt Solution	1X	02-015-1A 02-015-1B	500ml 100ml	AMB AMB
Hanks' Balanced Salt Solution Without Sodium Bicarbonate	10X	02-015-5A 02-015-5B	500ml 100ml	AMB AMB
Hanks' Balanced Salt Solution Without Phenol Red	1X	02-016-1A 02-016-1B	500ml 100ml	AMB AMB
Hanks' Balanced Salt Solution Without Calcium and Magnesium	1X	02-017-1A 02-017-1B	500ml 100ml	AMB AMB
Hanks' Balanced Salt Solution Without Calcium and Magnesium Without Phenol Red	1X	02-018-1A 02-018-1B	500ml 100ml	AMB AMB
Dulbecco's Phosphate Buffered Saline (DPBS)	1X	02-020-1A 02-020-1B	500ml 100ml	2-8°C 2-8°C
Dulbecco's Phosphate Buffered Saline (DPBS) Without Calcium and Magnesium	1X	02-023-1A 02-023-1B	500ml 100ml	AMB AMB
Dulbecco's Phosphate Buffered Saline (DPBS) Without Calcium and Magnesium	10X	02-023-5A 02-023-5B	500ml 100ml	AMB AMB
Spinner Modified Salt Solution	1X	02-030-1A	500ml	AMB
Elsever's Solution	1X	02-045-1A 02-045-1B	500ml 100ml	AMB AMB

See formulations on page 102

Cytokines and Growth Factors

5 Cytokines and Growth Factors

Cytokines are a large and diverse family of polypeptide regulators, signaling proteins and glycoproteins, which- like hormones and neurotransmitters- are critical to the development and functioning of both innate and adaptive immune response, as well as to other body systems. While hormones are secreted from specific organs to the blood and neurotransmitters are related to neural activity, cytokines are more of a diverse category or class of compounds in terms of origin and function.

These signaling molecules, historically known as immunomodulating agents (e.g. interferons, interleukins) like neurotransmitters, hormones and growth factors, function as mediators, directly and indirectly, in cellular communication by triggering specific cellular reactions or responses in target tissues and organs throughout many areas of the body, or to distinct, specific cells. Neurotransmitters act in a similar fashion when released at an axon terminal of a neuron that eventually locks on to a specific receptor site or target impacting another neuron, muscle or gland cell. Growth factors like cytokines are secreted by a variety of cells and bind to or lock on to specific high-affinity cell-surface target receptors, thereby eliciting similar or more specific biochemical changes through a cascade of events. Whereas the effects of cytokines include autocrine, endocrine and paracrine activity relative to target organ or cell-receptor site, growth factors not only elicit a cascade of events by participating in the regulation of cell growth and differentiation, but also by promoting replication and similar complex processes.

The cytokine family includes a variety of colony and growth-stimulating factors, interferons, interleukins, chemokines and other molecules that exhibit pleiotropic effects and considerable redundancy. They may act on the cellular level such as in cell differentiation, through tissue development, homeostasis, and in certain developmental processes, during embryogenesis. Due to the fact that they are characterized by such general effects and hence are often difficult to objectively characterize, such aforementioned distinctions, allowing for exceptions, are for the most part null and void. Nevertheless, this much is known about them. These distinct effects are multi-fold and depend upon, inter alia:

- The specific cytokine perse.
- The abundance or concentration of the cytokine relative to its location or target receptor.
- The complementary high-affinity receptor site or sites (presence and abundance).
- The quality of downstream signals triggered by the interaction of receptor binding activity.

For example, a variety of cytokines and growth factors influence the invasive properties of extravillous trophoblast and surrounding cells. Of these, Epidermal Growth Factor (EGF), Insulin-like Growth Factor II (IGF-II), Transforming Growth Factor β (TGF- β) and Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) and others have been

extensively studied in human implantation. Over fifty different proteins that function as growth factors have been isolated and identified with many more possibilities on the horizon. While some growth factors affect a broad range of cell types (e.g. EGF, PDGF) others are much more specific. Nerve Growth Factor (NGF), for instance, promotes the growth of certain classes of neurons, while erythropoietin stimulates red blood cell precursors triggering cell division. Most mammalian cells in culture need a combination of different growth factors and/or cytokines to impact the various controls that inhibit cell division or to elicit other more receptor-specific and distinct effects.

Biological Industries' Cytokines and Growth Factors are manufactured under strict and vigorous Quality Assurance Protocols and comply with verifiable product specifications. They are of the highest quality, exhibit high levels of bioactivity performance and are competitively priced.

Cytokines	Growth Factors
Adiponectin	Colony Stimulating Factor
Angiopoietin	CTGF
Apolipoprotein	Epidermal Growth Factor
B-cell Activating Factor	Erythropoietin
Beta Defensin	Fibroblast Growth Factor
Bone Morphogenetic Protein	Galectin
B type Natriuretic Peptide	Growth Hormone
Endoglin	Hepatocyte Growth Factor
Flt-3 Ligand	Insulin-Like Growth Factor
Hedgehog Protein	Insulin
Interleukin	Keratinocyte Growth Factor
Interferon	Leptin
Resistin	Macrophage Migration Inhibitory Factor
Thrombopoietin	Myostatin
Trefoil Factor	Noggin
Tumor Necrosis Factor	PDGF
Visfatin	Placental Lactogen
Other	Prolactin
	Stem Cell Factor
	Transforming Growth Factor
	VEGF
	Other

Custom Services

Custom Services

6

Contract Manufacturing
Custom Formulations

6 Custom Services

Contract Manufacturing

Biological Industries (BI) develop, manufacture, pack and supply biological products for academic research, diagnostic labs, hospitals and the biotechnology and biopharmaceutical industries. Our Contract manufacturing services range from raw material supply, through labeling and packaging to ready-to-market products, all to suit our customer's needs. Our extensive experience in liquid manufacturing and packaging makes partnering with BI an obvious choice as your products progress from the laboratory and pilot scales to full scale production.

Our tailored services include:

- Sterile filtration and aseptic filling.
- Flexible packaging in industrial single-use (bulk) bags from 0.5 to 2000 Liter.
- Research and pilot scale freeze-drying (lyophilization).
- Extensive range of Quality Control services.

BI manufacture under stringent quality system in a cGMP compliant, ISO 9001:2008 and ISO 13485:2003 certified facility. Our controlled environment clean rooms graded from ISO 8 up to ISO 5 (classified from class 100,000 to 100).

Having successfully undergone several recent audits, our clients are ensured of the highest quality products, along with:

- Consistent quality
- Rapid delivery
- Professional consultation
- Qualified personal
- Complete confidentiality.

Custom Formulations

Biological Industries specializes in the production of special formulation media and solutions exactly to the customer's specifications. The products are produced, sterile filtered and aseptically filled in dedicated clean rooms.

BI's "can-do" attitude and commitment to our customers' objectives is exemplified by:

- Small volume production, as small as 3 liters.
- Quick turnaround; within 15-20 days including QC.
- Flexible packaging.
- Dedicated experienced professionals.

Our tailored services include:

Modified Classical Cell Culture Media

Custom made media supplemented, substituted or devoid of specific components. One such example, are reagents without specific amino acids required for use in stable isotope labeling with amino acids (SILAC).

Customer-designed Media and Solutions

Custom-manufacturing of media and solutions according to the specification of an exact formulation and manufacturing process, provided by the customer. The solutions may be used for upstream and downstream purification processes (i.e. buffers).

Media Formulation Optimization

Our expert team of scientists determines the best available medium and experiments are carried out on an empirical basis. The team uses the latest techniques and is able to scale up the media modifications to a commercial level within a very short period.

All of BI's custom media may be QC tested for:

- Physical Appearance
- pH
- Osmolality
- Endotoxins
- Sterility
- Cell growth
- Conductivity

The following are examples of custom-made products:

Catalogue No.	Solution
01-010-1	BGJ- Fitton Jackson Modification
01-816-1	N2 Complete Medium, w/o Glutamine
01-832-1	MCDB 131
01-862-1	EDTA 0.5M

Catalogue No.	Solution
01-877-1	CRCM 30
01-971-1	Sodium Hydroxide (NaOH) 2.5M Solution
01-973-1	D-Glucose 20% Solution
02-030-1	Spiner Modified Salt Solution
03-095-1	Chicken Embryo Extract
06-1025-03-1	MEM-E w/o P.R. Methionine
06-1025-22-1	MEM-E Salts w/o Glucose
06-1025-25-1	MEM Eagle with Hepes
06-1042-14-1	MEM Alpha, Low Glucose, w/o Ascorbic Acid
06-1042-16-1	MEM Alpha, with 2mM Glutamine, 1.5g/l Bicarbonate
06-1043-01-1	MEM Alpha w/o Glucose
06-1050-17-4	DMEM, w/o Glucose, Pyruvate 5X
06-1055-04-1	DMEM, High Glucose
06-1055-08-1	DMEM, High Glucose, w/o Valine
06-1055-09-1	DMEM, High Glucose, with D-Valine
06-1055-33-1	DMEM, High Glucose, w/o Histidine
06-1055-38-1	DMEM, High Glucose, 25mM Hepes
06-1055-45-1	DMEM, High Glucose, 25mM Hepes, w/o Glutamine & Phenol Red
06-1055-49-1	DMEM, High Glucose, w/o Lysine
06-1055-57-1	DMEM, High Glucose, with Glutamine, Sodium Pyruvate
06-1055-66-1	DMEM, High Glucose, w/o Amino Acids
06-1055-68-1	DMEM, High Glucose, w/o Folic Acid
06-1055-70-1	DMEM, High Glucose, w/o Lysine, Arginine
06-1055-74-1	DMEM, High Glucose, w/o Lysine, Arginine, Leucine
06-1055-75-1	DMEM, High Glucose, w/o Leucine & L-Valine
06-1055-77-1	DMEM, High Glucose, w/o Amino Acids & Glucose
06-1058-16-1	IMDM with 4mM Glutamine, 1.5g/l Bicarbonate
06-1060-02-1	Glasgow's with Glutamine, with Tryptose Phosphate Broth
06-1075-05-1	SILAC MCCOYS 5A, w/o Lysine, Arginine, Glutamine
06-1080-15-1	M-199 Earle's w/o Glucose & Amino Acids
06-1080-18-1	M-199 Earle's w/o Sodium Bicarbonate
06-1095-02-1	F-12 (HAM) Coon's Modification
06-1095-13-1	F-12 (HAM), w/o Glutamine

Catalogue No.	Solution
06-1095-15-1	HITES Medium (F-12K+DMEM)
06-1095-16-1	F12 K with 1.5J/l Sodium Bicarbonate
06-1095-22-1	F-12 with Alanyl-Glutamine
06-1095-28-1	F-12 w/o Folic Acid
06-1100-06-1	RPMI-1640 w/o Folic Acid
06-1100-07-1	RPMI-1640 w/o Arginine
06-1100-26-1	RPMI-1640 w/o Ribof., Phenol Red
06-1100-38-1	RPMI-1640, with Glutamine, w/o Calc. & Mg.
06-1100-39-1	RPMI-1640 w/o Phenylalanine & Tryptophan
06-1104-32-1	RPMI-1640, w/o Glutamine & Tryptophan
06-1106-12-1	RPMI-1640, with Hepes, Sodium Pyruvate
06-1150-05-1	Schneider w/o Arginine, Lysine
06-1170-02-1	DMEM:F-12, w/o Glucose
06-1170-15-1	DMEM:F-12, w/o Phenol Red, w/o Glutamine
06-1170-19-1	DMEM:F-12, 3:1, with Glutamine, w/o Calcium Bicarbonate
06-1170-23-1	DMEM:F-12, with Glucose 1g/l
06-1170-34-1	DMEM:F-12, with L-Glutamine
06-1170-48-1	DMEM:F-12, with 2% BSA
06-1170-50-1	DMEM:F-12, 1:1, w/o Hepes, with 2.4g/l Sodium Bicarbonate
06-1170-51-1	DMEM:F-12, with 1gr/l D-Glucose & 2% BSA
06-1170-52-1	DMEM:F-12, w/o Phenylalanine, Tryptophan
06-1170-53-1	DMEM:F-12, with 2mM Glutamine, w/o Glucose, Phenol Red
06-1802-05-9	Modified MCDB 153 Conc.X2
06-1802-08-1	Modified MCDB 153
06-1821-02-1	CMRL 1066 w/o Phenol Red
06-2010-01-1	Earle's BSS, w/o Glucose
06-2010-02-1	Earle's Balanced Salt Solution
06-2023-01-1	PBS w/o Cl, Mg, with EDTA
06-3022-01-1	L-Alanyl L-Glutamine, 400mM

Price quotations for all of the above services are available upon request. For an immediate quote, please contact Biological Industries at: Tel: 972-4-9960-595 or by fax: 972-4-996-8896

Sera

7

Foetal Bovine Sera (FBS)

Bovine Sera

Other Sera

7 Sera

7

Sera

Introduction

Serum is commonly used as a supplement to basal growth medium in cell culture. The most common type of serum used for cell growth is foetal bovine serum (FBS), also known as foetal calf serum (FCS). Foetal bovine serum is obtained from foetuses harvested in abattoirs from healthy dams fit for human consumption.

Occasionally, there may be use of other bovine sera, such as newborn calf serum or donor bovine serum. In cell culture, serum provides a wide variety of macromolecular proteins, low molecular weight nutrients, carrier proteins for water - insoluble components, and other compounds necessary for in vitro growth of cells, such as hormones and attachment factors. Serum also adds buffering capacity to the medium and binds or neutralizes toxic components. Attempts to replace serum entirely with serum-free medium have met only with limited success. The selection of a serum supplement for cell culture applications is primarily dependent on the chemical definition of the basal medium, the type of cell to be grown, and the culture system being employed.

Collection

In the FBS manufacturing process, whole blood is collected aseptically in disposable sterile plastic bags and allowed to clot. Once the serum has been separated from the clot, it is pooled and frozen.

Controlling the initial collection of foetal blood is a crucial factor in the quality of the final serum product. Only raw material that meets our specifications is approved for production.

Handling Serum Products

Using Serum – Although the product has been sterile filtered, aseptic procedures must be followed at all times.

Granules, flocculent material or turbidity may develop after thawing. This particulate matter does not alter the performance of the serum as a supplement for cell culture medium. Repeated freezing/thawing of serum may increase the amount of precipitate and is therefore not recommended. If you do not intend to use an entire bottle of serum aliquot it into usable quantities in sterile containers before freezing a second time.

Wipe the outside of each bottle with a suitable disinfectant solution, before setting it on the work surface. Remove the heat seal and wipe the outside of the cap with the disinfectant solution.

All serum products should be treated as potentially harmful and appropriate care should be taken when handling them.

Raw Materials

Two distinct grades of FBS are available on the world market: USDA-Grade FBS and European-Grade FBS.

USDA-Grade FBS is produced from raw materials originating only from countries certified to be free of both BSE (Bovine Spongiform Encephalopathy) and FMD (Foot and Mouth Disease). This product can be freely imported into any country, and is the product of choice in all countries for manufacturing purposes. Furthermore, only the use of this product allows researchers to send their cells, or the products of their cells, to collaborators in other countries with strict import regulations.

All FBS processed in the Biological Industries plant is USDA-Grade.

Processing

Selected batches of serum raw material are thawed, tested for endotoxins and hemoglobin content and only the accepted material is pooled. The pooled raw material is thoroughly blended under refrigerated conditions and membrane filtered for sterility according to a well validated filtration protocol. Biological Industries processes FBS through a sequence of pre-filters and membrane filters. The filtration step includes the use of three 0.1 micron sterilizing grade membrane filters in series.

After filtration, the serum is dispensed into bottles by an aseptic filling process which has been validated to insure sterility of the final product. Serum products are produced in a controlled environment (clean rooms) designed to carefully control air pressure and particulate matter.

The manufacturing area is a class 100,000 (ISO 8) environment. The sterile bottles and equipment are stored in a class 10,000 (ISO 7) environment, and the filling room is a class 1000 (ISO 6) environment with class 100 (ISO 5) laminar air flow sterile bench.

Clean rooms are monitored on a regular basis for particulate and microbial levels to ensure that the air handling system, cleaning protocols and personnel maintain standards control.

After filling, the final product is quickly frozen to -20°C and held in quarantine until all quality control tests have been completed.

Quality Control

Each lot of FBS is tested to confirm that the serum meets the written specifications. Final product release is done after reviewing all production and quality control records to determine compliance with all established, approved written procedures.

Physical and chemical tests

- Electrophoretic Pattern
- pH
- Osmolality
- Total proteins
- Albumin
- IgG
- Hemoglobin
- Globulins

Biochemicals Tests

The following tests are conducted on each lot of FBS:

- Alanine Transaminase (ALT)
- Alkaline Phosphatase
- Aspartate Aminotransferase (ast)
- Bilirubin – total
- Bilirubin – direct
- Blood Urea Nitrogen (BUN)
- Calcium
- Chloride
- Cholesterol
- Creatinine
- Creatinine Kinase (CK)
- Gamma-Glutamyl Transferase (GGT)
- Glucose
- High Density Lipoproteins (HDL)
- Lactate Dehydrogenase
- Low density Lipoproteins (LDL)
- Phosphorous (Inorganic)
- Potassium
- Sodium
- Triglycerides (TG)
- Uric Acid

Microbiological tests

- **Sterility tests:**
Bacterial and fungal sterility tests according to the current USP
- **Mycoplasma contamination:**
According to the Code of Federal Regulations (CFR), title 9, part 113 (culture method).
- **Viral contaminants:**
According to the protocols described in CFR, title 9, part 113 for Bovine Viral Diarrhea (BVD), Infectious Bovine Rhinotracheitis (IBR) and Parainfluenza type 3 (PI3).
- **Viral antibodies:**
FBS is screened to determine the titer of neutralizing antibodies to BVD, IBR and PI3.
- **Endotoxins:**
The test is performed using the standard Limulus Amebocyte Lysate (LAL) with the kinetic turbidimetric method.

Biological performance (cell growth)

The cell growth tests are designed to check the efficacy of the FBS in promoting cell growth. Cells used are fibroblasts (MRC-5 diploid normal cells), epithelial cells Vero and hybridoma cells. Each test is conducted using the tested serum and a validated control lot. Growth promotion using MRC-5 cells is evaluated through several subculture generations to observe any evidence of cytotoxicity and morphological changes of the cells.

Vero cells (ATCC, CCL 81): plating efficiency.

MRC-5 cells (ATCC, CCL 171): 3 passages test.

Hybridoma cells: cell growth.

Stability

FBS stability at -20°C temperature was evaluated with several cell types for long periods. The FBS did not lose its performance for 55 months (4.5 years) with all the cells tested. Storage of FBS at -20°C without defrosting will maintain the quality of the FBS at least until the expiration date stated on the label.

Quality Assurance

The FBS production process is carried out under controlled conditions in a controlled environment. The steam-in-place (SIP) sterilization, filtration for sterility and filling are validated as required for key aseptic processes. A dossier (Device Master Record) exists for serum with all relevant data concerning serum production. The production process from the raw material to the final product in storage, as well as the quality control tests and results, are documented and filed to ensure traceability and control of the process.

Biological Industries' products are manufactured in compliance with the quality management standard ISO 9001:2008 and ISO 13485:2003. Certifications are available upon request.

In addition, the FBS production process conforms to the In Vitro Diagnostics Directive (IVDD 78/79/EC) of the European Parliament. Therefore, our FBS received the CE mark making it eligible for sale in the European Union for in vitro diagnostics.

A Bovine Spongiform Encephalopathy (BSE) Certificate of Suitability has been issued to Biological Industries by the European Directorate for the Quality of Medicines (EDQM) in accordance with monographs of the European Pharmacopeia.

BI is also a member of the International Serum Industry Association (ISIA). All documents and certifications are available upon request.

Foetal Bovine Sera (FBS)

Serum, as a biological material, represents an undefined mixture of components in which composition varies from one lot to the other. Some cell types are sensitive to the variations in serum performance. Customers are encouraged to evaluate serum samples with their own culture system and cells while we reserve the quantities of the specific lots until customer testing is completed. In this way, the customer may choose the best serum for his own applications.

Biological Industries offers you the following certified sterile Foetal Bovine Serum Products:

Product Name	Catalogue No.	Unit Size	Storage Temp.
Certified Foetal Bovine Serum	04-001-1A	500ml	-20°C
	04-001-1B	100ml	-20°C
Certified Foetal Bovine Serum Heat Inactivated	04-121-1A	500ml	-20°C
	04-121-1B	100ml	-20°C
Certified Foetal Bovine Serum Qualified for Human Embryonic Stem Cells	04-002-1A	500ml	-20°C
	04-002-1B	100ml	-20°C
Certified Foetal Bovine Serum Qualified for Human Embryonic Stem Cells Heat Inactivated	04-222-1A	500ml	-20°C
	04-222-1B	100ml	-20°C
Certified Foetal Bovine Serum Functionally Tested for use with Tetracycline Regulated Systems	04-005-1A	500ml	-20°C
	04-005-1B	100ml	-20°C
Certified Foetal Bovine Serum Functionally Tested for use with Tetracycline Regulated Systems Heat Inactivated	04-125-1A	500ml	-20°C
	04-125-1B	100ml	-20°C
Certified Foetal Bovine Serum Dialyzed	04-011-1A	500ml	-20°C
	04-011-1B	100ml	-20°C
Certified Foetal Bovine Serum, Charcoal-Stripped	04-201-1A	500ml	-20°C
	04-201-1B	100ml	-20°C
Certified Foetal Bovine Serum (FBS), Qualified for Mesenchymal Cells	04-400-1A	500ml	-20°C
	04-400-1B	100ml	-20°C
Certified Foetal Bovine Serum (FBS), Gama Irradiated	04-111-1A	500ml	-20°C

Heat inactivated FBS

Product Name	Catalogue No.	Unit Size	Storage Temp.
Certified Foetal Bovine Serum Heat Inactivated	04-121-1A	500ml	-20°C
	04-121-1B	100ml	-20°C

Heat inactivation of serum is performed by raising the temperature of the serum to 56°C and maintaining that temperature for 30 minutes. Heat inactivation is the method of choice to destroy complement proteins activity.

FBS qualified for human Embryonic Stem (ES) Cells

Product Name	Catalogue No.	Unit Size	Storage Temp.
Certified Foetal Bovine Serum Qualified for Human Embryonic Stem Cells	04-002-1A	500ml	-20°C
	04-002-1B	100ml	-20°C
Certified Foetal Bovine Serum Qualified for Human Embryonic Stem Cells Heat Inactivated	04-222-1A	500ml	-20°C
	04-222-1B	100ml	-20°C

Embryonic Stem (ES) Cells are pluripotent cells derived from the inner cell mass of the blastocyst. The stem cells can be maintained in vitro for extended periods without loss of their capacity to differentiate to all cell lineages when reimplanted back into a blastocyst. ES cells may differentiate in vitro to a variety of cell types including neuronal, muscle, endothelial and hematopoietic progenitors. General culture conditions are well established and usually require ES cells to be grown on an inactive feeder cell layer or with basement membrane components (Matrigel, Fibronectin, Laminin). When growing ES cells, one of the most important parameters is the maintenance of the cells in the undifferentiated state. Pre-screening of the serum is essential before using it for the culture of ES cells. Various lots of sera are screened for the growth of Human ES cells using MEF's as a feeder layer.

The following parameters are measured for the screening:

- Human ES cells colony morphology
- Plating efficiency
- Differentiation rate: analysis of Human ES cells surface marker expressed on the undifferentiated cells membrane (FACS analysis). The results are used as an indication of the quality of sera for the growth and maintenance of undifferentiated stem cells.

FBS Qualified for Mesenchymal Stem Cells

Product Name	Catalogue No.	Unit Size	Storage Temp.
Certified Foetal Bovine Serum (FBS) Qualified for Mesenchymal Stem Cells	04-400-1A	500ml	-20°C
	04-400-1B	100ml	-20°C

Mesenchymal Stem Cells, also known as MSC's, are another well-characterized population of adult stem cells. These are multipotent stem cells that can differentiate into a variety of cell types and include chondrocytes, myocytes, adipocytes, and, as described recently, betapancreatic islets cells. Although not immortal, they have the ability to expand exponentially in culture while retaining their growth and multilineage potential. Unlike most other Human Adult Stem Cells, MSC's can be obtained in quantities appropriate for clinical applications, making them good candidates for use in tissue repair. The established techniques utilized for the isolation and amplification of MSC's in

culture have proven that these types of cells can be maintained and propagated in cell culture for the long-term without losing their viability and their capacity to differentiate. Furthermore, MSC's can take up and keep introduced genes, a phenomenon that may be exploited for the delivery of advantageous molecules to targeted sites.

Perhaps one of the most exciting considerations for Human applications is that MSC's may be derived from a small bone marrow sample from a given patient, expanded in culture and given back to the patient, thus avoiding the typical problems associated with immune rejection of foreign transplanted cells or tissues.

Pre-screening of the serum is an essential procedure before using it for culture of MSC's as Stem Cell Research requires the highest possible serum purity to prevent adverse differentiation. Biological Industries pre-screens multiple FBS batches/lots to eliminate the often time-consuming and costly process of testing and re-testing in order to allow researchers to focus on more important tasks. Various Lots/Batches of Sera are screened for the growth of Mesenchymal Stem Cells.

The following parameters are measured during the screening process:

- MSC Morphology
- Plating Efficiency
- Differentiation Rate

The results are utilized as an indication of the highest purity of the sera that emphasizes maximum expansion of unaltered MSC's, while at the same time, maintaining their unrestricted multipotency.

FBS - Functionally Tested for use with Tetracycline Regulated Systems

Product Name	Catalogue No.	Unit Size	Storage Temp.
Certified Foetal Bovine Serum	04-005-1A	500ml	-20°C
Functionally Tested for use with Tetracycline Regulated Systems	04-005-1B	100ml	-20°C
Certified Foetal Bovine Serum	04-125-1A	500ml	-20°C
Functionally Tested for use with Tetracycline Regulated Systems Heat Inactivated	04-125-1B	100ml	-20°C

Functionally Tested for use with Tetracycline Regulated Systems FBS that have been pre-tested to ensure that they permit the range of tetracycline-regulated induction with well-characterized Tet lines.

Dialyzed FBS

Product Name	Catalogue No.	Unit Size	Storage Temp.
Certified Foetal Bovine Serum Dialyzed	04-011-1A	500ml	-20°C
	04-011-1B	100ml	-20°C

Most cells grown in culture require a serum component of the growth medium to maintain their proliferative capacity. While whole serum is permissible for routine purposes, studies involving nutritional parameters or incorporation of labeled material require that the constituent under study be removed from the serum. The most commonly used method for removal of these constituents is dialysis of whole serum.

For dialysis by diafiltration, serum is circulated through a hollow-fiber by the concentration method. The filtrate, however, is replaced by the addition of physiological saline to the serum.

Charcoal-stripped FBS

Product Name	Catalogue No.	Unit Size	Storage Temp.
Certified Foetal Bovine Serum	04-201-1A	500ml	-20°C
Charcoal-Stripped	04-201-1B	100ml	-20°C

Charcoal-stripped FBS is used to elucidate the effects of hormones in a variety of in vitro systems. Studies include steroid- receptor binding, steroid regulation of cellular receptors, hormone secretion of various tissues and the function of thyroid hormones. The production procedure includes the use of charcoal and dextran to remove the hormones from the FBS.

Gama irradiated FBS

Product Name	Catalogue No.	Unit Size	Storage Temp.
Certified Foetal Bovine Serum, (FBS) Gama Irradiated	04-111-1A	500ml	-20°C

Gamma irradiation is a process which involves exposing material to high energy gamma photons released by radioisotopes, such as cobalt 60. This energy is transferred from the photons to the products, and is responsible for organism inactivation by ionization of nucleic acid bonds. The irradiation of serum is intended to provide complete assurance of viral inactivation. An extensive validation study has been performed to validate the irradiation process using FBS spiked with several viruses. We have demonstrated that properties and cell culture performance of FBS are not altered by gamma irradiation exposure up to 3.5 MRad.

Foetal Bovine Sera (FBS) FAQs

- What is the difference between Foetal Bovine Serum (FBS) and Foetal Calf Serum (FCS)?**
 They are the same and describe exactly the same serum product.
- My FBS contains precipitates. What are they and what should I do?**
 The precipitates contain fibrin (clot forming protein) and lipoproteins. This is a normal characteristic and will not affect product performance. To remove the precipitates, centrifuge the serum or simply let it settle to the bottom of the bottle and transfer the serum carefully to another sterile bottle.
- My FBS arrived partially thawed, what should I do?**
 Let the serum defrost completely, swirl the serum bottle gently and then re-freeze the serum. The quality of the serum will not have been affected.
- How do I heat-inactivate serum?**
 Serum heat-inactivation is performed in a waterbath at 56°C for 30 minutes. The water level should be higher than the level of the serum. Monitor the temperature in a reference bottle containing water at the same volume during the heat inactivation. You must swirl the bottles to mix the serum every 5 minutes during heat inactivation to insure uniform heating. Use a calibrated thermometer only!
- I have a jellylike fraction on the bottom of the bottle. What is it?**
 As a result of improper heat inactivation of the serum (temperature above 56°C, more than 30 minutes), or inactivation without mixing the serum, a protein denaturation caused the jellylike fraction on the bottom of the bottle. Do not use this serum.
- How should I thaw FBS?**
 We recommend thawing the serum at 2-8°C. However, if necessary, you may thaw the serum at room temperature. Swirl the bottles gently to mix the serum during the thawing process.
- Why is the color of the FBS not exactly the same as with my previous lot?**
 The color of FBS is brown to brown-red. It is dependent mainly on the hemoglobin concentration in the specific lot. The color does not affect the FBS performance.
- Is the FBS raw material free of BSE?**
 FBS sold by Biological Industries has always been produced from raw materials originating from countries that are free of BSE according to the OIE (Office International des Epizooties).
- How can I be certain regarding the country of origin of the raw material that was used to manufacture sterile FBS?**
 Ask Biological Industries for a copy of the original raw material veterinary documents.

Bovine Sera

Newborn Calf Sera

Product Name	Catalogue No.	Unit Size	Storage Temp.
Newborn Calf Serum (Less than 10 days)	04-102-1A	500ml	-20°C
	04-102-1B	100ml	-20°C
Newborn Calf Serum Heat Inactivated	04-122-1A	500ml	-20°C
	04-122-1B	100ml	-20°C

Bovine Sera

Product Name	Catalogue No.	Unit Size	Storage Temp.
Adult Bovine Serum	04-003-1A	500ml	-20°C
	04-003-1B	100ml	-20°C
Adult Bovine Serum Heat Inactivated	04-123-1A	500ml	-20°C
	04-123-1B	100ml	-20°C

Other Sera

Product Name	Catalogue No.	Unit Size	Storage Temp.
Donor Horse Serum	04-004-1A	500ml	-20°C
	04-004-1B	100ml	-20°C
Donor Horse Serum Heat Inactivated	04-124-1A	500ml	-20°C
	04-124-1B	100ml	-20°C
Porcine Serum	04-006-1A	500ml	-20°C
	04-006-1B	100ml	-20°C
Rabbit Serum	04-008-1A	500ml	-20°C
	04-008-1B	100ml	-20°C
Donor Goat Serum	04-009-1A	500ml	-20°C
	04-009-1B	100ml	-20°C

Mycoplasma Detection and Treatment

Mycoplasma Detection and Treatment

8

8 Mycoplasma Detection and Treatment

Mycoplasma Detection

Mycoplasma is a prokaryotic microorganism of the class Mollicutes that lack a true cell wall, and many of which are considered pathogenic. Mycoplasma contamination is often detected in cell cultures, and consequently, virus cultures, vaccines and other biological materials produced in cells become contaminated as well.

Mycoplasma contamination in cell lines used for research poses a serious problem. In most cases, visual detection of such contaminations or detection with the aid of a microscope is impossible. Although mycoplasma does not cause visible damage to cells, it undeniably affects cell metabolism, cell growth in culture, protein synthesis, cytokine secretion, and even causes damage to DNA and RNA. Hence, results obtained from experiments are liable to be biased when mycoplasma is present. Various studies show that the percentage of contaminated cultures in cell banks is 10%-80%. Mycoplasma contamination can originate from bovine serum, laboratory employees, other contaminated cultures, or the animals from which the cells have been harvested.

The most prevalent species of mycoplasma detected in contaminated cell cultures include *M. fermentans*, *M. hyorhinis*, *M. arginini*, *M. orale*, *M. salivarium*, *M. hominis*, *M. pulmonis* and *M. pirum*.

Testing Methods

Several methods for the detection of mycoplasma have been published:

- Cultures on agar, liquid media, or semi-solid media.
DAPI Staining – staining DNA with fluorescent dyes (4', 6-diamine-2 phenylindole dihydrochloride).
- DNA hybridization.
- Antibodies for specific mycoplasma species.
- Electronic microscope.
- PCR: specific primers.
- Biochemical – detection of mycoplasmal enzymes by colorimetric or luminescence assay.

Using PCR for the Detection

The testing required by the regulatory authorities is seeding in culture (agar and liquid media). This test is complicated, time consuming (about 5 weeks), and some mycoplasma species are difficult to detect with this method. In recent years, the disadvantages of these methods have been acknowledged (such as sensitivity, specificity and long and complex procedures), and use of PCR for the detection of contaminations in cell cultures has become increasingly widespread.

PCR has been shown to be a highly sensitive, specific and rapid method for the detection of mycoplasma contamination in cell cultures. Specific primers have been designed from DNA that is coded to the ribosomal RNA (16SrRNA). The gene sequences for RNA are considered conserved sequences and are similar in the various mycoplasma species, which have not undergone significant mutation. Consequently, primers can be designed for these areas, which are specific to the mycoplasma and will not detect bacterial or animal DNA sequences.

The literature describes several PCR methods for the detection of mycoplasma, such as using a number of primers to obtain detection of specific mycoplasma species, and nested PCR (two consecutive PCR cycles using different primers) for amplifying sensitivity and specificity. PCR testing techniques are based on amplification of a DNA fragment using primers prepared in advance, and fragment identification is usually carried out with electrophoresis.

In conjunction with Prof. Shlomo Rottem of the Mycoplasma Laboratory at the Hebrew University-Hadassah Medical School, Jerusalem, Biological Industries has developed the EZ-PCR Mycoplasma Test Kit (Cat. # 20-700-20) a PCR-based mycoplasma test kit that simplifies testing and detection of mycoplasma contamination in cell cultures. The kit includes a unique reaction mix that contains all the ingredients required for PCR: nucleotides, primers, Taq Polymerase and magnesium. No prior preparations are required for PCR, other than the sample to be tested (centrifugation and suspension in the buffer supplied with the kit). After performing agarose gel electrophoresis, positive samples will yield a 270bp fragment. The test takes approximately five hours to complete.

The primers have been designed to detect the mycoplasma species responsible for most contaminations in cell cultures (including *Acholeplasma*). The primers were tested and found to be specific to mycoplasmatic DNA, and do not react with animal or bacterial DNA.

In sensitivity tests for the detection of defined mycoplasmas, the EZ-PCR Mycoplasma Test Kit was found to be very sensitive in comparison to other test kits currently available on the market (Table 1). The ability to routinely conduct rapid and simple tests to detect mycoplasma contamination in cell cultures facilitates the eradication or treatment of contaminated cells.

Table 1: Minimal concentration of mycoplasma detected with EZ-PCR Mycoplasma Test Kit

	Without Sample Preparation	After Sample Preparation (conc. 1/20)*
<i>M. fermentans</i>	240 CFU/ml	12.00 CFU/ml
<i>M. capricolum</i>	110 CFU/ml	5.50 CFU/ml
<i>M. penetrans</i>	200 CFU/ml	16.66 CFU/ml
<i>M. hyorhinis</i>	210 CFU/ml	10.50 CFU/ml

* According to EZ-PCR Mycoplasma Test Kit instructions.

EZ-PCR Mycoplasma Test Kit

Product Name	Catalogue No.	Unit Size	Storage Temp.
EZ-PCR Mycoplasma Test Kit	20-700-20	20 assays	-20°C

Ready-to-use PCR Mix for the detection of mycoplasma in cell culture

EZ-PCR Mycoplasma Test Kit is designed to detect the presence of mycoplasma in contaminated biological materials, such as cultured cells. Mycoplasma detection by the direct culture procedure is time-consuming and some mycoplasma species are difficult to cultivate. With PCR testing, results are obtained within a few hours, since the presence of contaminant mycoplasma can be easily detected simply by verifying the bands of amplified DNA fragments in electrophoresis. There is no need to prepare probes labeled with radioisotopes, or to calculate enzyme, dNTPs or buffer concentrations. Instead, a ready-to-use, optimized PCR mix is supplied. The reaction mix contains a precipitant for direct loading of PCR products onto agarose gel. The primer set allows detection of various mycoplasma species (*M. fermentans*, *M. hyorhinitis*, *M. arginini*, *M. orale*, *M. salivarium*, *M. hominis*, *M. pulmonis*, *M. arthritis*, *M. bovis*, *M. pneumoniae*, *M. pirum* and *M. capricolum*), as well as *Acholeplasma* and *Spiroplasma* species, with high sensitivity and specificity.

Advantages

EZ-PCR Mycoplasma Test kit is based on a simple assay protocol and has the following advantages:

- Highly sensitive.
- Mycoplasma-specific primers with broad range.
- Convenient and user-friendly: supplied with complete reaction mix (with Taq polymerase). Requires no more than 10-20 minutes of actual work.
- Samples are easy to prepare.
- Results are easily determined with a single PCR process.
- Rapid: results obtained in no more than 5 hours.
- No loading dye needed for the agarose gel.

Kit Components

Reaction Mix: 200 µl

Buffer Solution: 1ml

Positive Template Control: 20µl

Principle

rRNA gene sequences of prokaryotes, including mycoplasmas, are well conserved, whereas, the lengths and sequences of the spacer region in the rRNA operon (for example the region between 16S and 23S gene) differ from species to species.

The detection procedure utilizing the PCR process with this primer set consists of:

1. Amplification of a conserved and mycoplasma-specific 16S rRNA gene region using two primers.
2. Detection of the amplified fragment by agarose gel electrophoresis. This system does not allow the amplification of DNA originating from other sources, such as tissue samples or bacteria, which affect the detection result. Amplification of the gene sequence with PCR using this primer set enhances not only the sensitivity, but also the specificity of detection. Amplified products are then detected by agarose gel electrophoresis.

Mycoplasma Treatment

Using Antibiotics to Disinfect Cell Cultures

The increasingly widespread use of more sophisticated and sensitive methods for the detection of mycoplasma contamination in cell cultures has resulted in contamination being detected in numerous cultures. This raises the issue of how to eliminate mycoplasma contamination. Naturally, the ideal solution is to discard the contaminated cells. However, if the cells that are stored in liquid nitrogen are also contaminated, a solution is required for eliminating the mycoplasma and preparing a new cell bank, particularly if the cells are unique and the result of extensive work.

A number of effective methods for the elimination of mycoplasma contamination in cell cultures have been published, such as:

- Treatment with specific hyperimmune serum (antibodies).
- Passage of contaminated cells in thymus-deficient mice.
- Exposure to analogs of nucleic acids that prevent reproduction of mycoplasma.
- Treatment with antibiotics.
- Exposure of contaminated cells to mouse macrophages.
- A technique that combines growing cells on soft agar and treatment with antibiotics.

The preferred method in terms of simplicity is treatment with antibiotics, which do not damage or alter cells. Antibiotics such as penicillin, which attacks bacterial cell walls, are ineffective in this instance, since mycoplasma lacks a true cell wall. Several antibiotics eliminate mycoplasma effectively, such as Tylosin, Neomycin, Tetracycline and Gentamycin. However, the efficacy of these antibiotics is restricted to specific mycoplasma species and frequently only reduces the concentration of mycoplasmas, rather than disinfect the cell culture. Consequently, as soon as treatment is concluded, contamination will recur.

Two methods are recommended for treating contaminated cells with antibiotics. The first is based on alternating treatment with two types of antibiotics (Tiamutin and Minocycline), and the second on treatment with one type of antibiotic (Ciprofloxacin).

Summary

Heightened awareness regarding mycoplasma contamination, and increased use of sensitive and effective methods for the detection and treatment of mycoplasma contaminations, will lead to a reduction in the percentage of contaminated cultures. In addition to isolating contaminated cultures, and discarding or treating them, meticulous work procedures should be followed, and only mycoplasma-free raw materials should be used.

The contamination of cells with mycoplasma is a very common problem, although it often goes unnoticed since no cloudiness appears in the cell culture. Nevertheless, the contamination often causes biochemical changes as well as changes in the immunological properties of the cells. Since mycoplasma-infected cells cannot always be discarded, many complex methods have been suggested for the elimination of mycoplasma.

Biological Industries is now offering a combination of antibiotics, which have been shown to be effective in the elimination of mycoplasma species that account for 90% of the contamination found in cell cultures. When used according to the following instructions, no cytotoxic effects will occur.

BIOMYC-1 & BIOMYC-2

Product Name	Catalogue No.	Unit Size	Storage Temp.
BIOMYC-1 Antibiotic Solution 100X Conc.	03-036-1B	100ml	-20°C
	03-036-1C	20ml	-20°C
	03-036-1D	10ml	-20°C
BIOMYC-2 Antibiotic Solution 100X Conc.	03-037-1B	100ml	-20°C
	03-037-1C	20ml	-20°C
	03-037-1D	10ml	-20°C

BIOMYC-1 is based on the antibiotic Tiamutin, which is produced by the fungus *pleurotus mutilus*. BIOMYC-2 is based on minocycline, which is a tetracycline derivative. These two antibiotic solutions are generally used sequentially in combination.

Instructions for Use

1. Do not use the two solutions together, but sequentially.
2. Add 1ml BIOMYC-1 to 100ml medium, and maintain the contaminated cells in this mixture for 4 days. Any fresh medium added should also contain BIOMYC-1.
3. After 4 days, add 1ml BIOMYC-2 to 100ml fresh medium, and maintain the cells in this second mixture for 3 days.
4. The above, together, are considered as one treatment cycle. It may be necessary to repeat this cycle 2-3 times.
5. During the process, the cells can be tested for mycoplasma contamination, and results can then be used to shorten the process when possible.

BIOMYC-3

Product Name	Catalogue No.	Unit Size	Storage Temp.
BIOMYC-3 Antibiotic Solution 100X Conc.	03-038-1B	100ml	-20°C
	03-038-1C	20ml	-20°C
	03-038-1D	10ml	-20°C

BIOMYC-3 is based on the ciprofloxacin antibiotic, which is a member of the fluoroquinolone group. Many mycoplasma species have been found to be sensitive to BIOMYC-3, including *A.laidlawii*, *M. orale*, *M.hyorhinae*, *M.fermentans* and *M.arginini*. These species are responsible for most of the contamination in cell culture⁽¹⁾. At the recommended concentrations, no cytotoxic effects have been found, and the treatment is quite easy to perform.

Instructions for Use

1. Add 1ml BIOMYC-3 to 100ml medium.
2. Continue the treatment for a total of 14 days, changing the medium (containing BIOMYC-3) every 2-3 days.
3. Retain the cells in the growth medium for an additional 14 days before re-testing for mycoplasma.

⁽¹⁾ Schmitt, k. et al., J. Immunol. Methods, 109: 17-25 (1988)

Disinfectants

Disinfectants

9

9 Disinfectants

The term 'disinfection' as denoted here refers to the destruction of potentially pathogenic microorganisms on inanimate objects. Disinfectants are basically chemical agents that are either lethal to microorganisms or inhibit their microbial activity and growth. Chemical disinfection plays a very crucial role, especially in the laboratory setting. Microorganisms, the cause of infectious diseases, are a heterogeneous group of organisms with a wide range of characteristics. With such a wide spectrum of biological characteristics, it is to be anticipated that particular groups of microorganisms or strains within a group will respond differently to the various means of disinfection. Disinfection is only one of the variegated measures that can be utilized to prevent and/or break the cycle of disease, but if used alone it is often adequate. Good hygiene and sanitation practices within the work setting are essential if the disinfection process is to be effective.

Some of the modes of action of disinfectants include:

- Adversely affecting the microbe's cellular physical structure by disruption of intermolecular interactions.
- Causing ensuing dissociation of the cellular bilayers .
- Compromising cellular permeability and/or
- Inducing leakage of cellular contents by enzymatic dissociation.
- Deactivation of other cellular metabolic processes.

An understanding of the basic principles involving the disinfection process is essential to achieve a satisfactory outcome. The selection and use of any chemical disinfectant requires a detailed knowledge of such factors, such as:

- Safety (i.e., potential hazards and toxicity)
- Range/spectrum of activity
- Ambient room temperature and humidity
- Type of surface
- Effectiveness in the presence of organic matter, lipids, fatty-acids and proteins.
- Stability and reactivity
- Neutralization by pH changes
- Neutralization by soaps or detergents
- Water hardness
- Contact time
- Specificity
- Environmental considerations

The aim of chemical disinfection is not to sterilize surfaces but rather to reduce the extent of microbial contamination to the lowest possible level. Most people utilizing disinfectants appreciate how essential it is to practice pristine hygiene and sanitation protocols, especially in a laboratory setting. Remember that disinfectants do not act instantaneously. Destruction of pathogens occurs in three phases:

1. **Initial/Lag Phase** - When the disinfectant starts showing activity
2. **Median Phase** - When the majority, but not all the microorganisms might be killed
3. **Final Phase** - When the more resistant microorganisms are destroyed.

As a rule, allow the disinfection process the necessary time to optimize its biocidal activity.

Biological Industries offers a variety of effective disinfectant agents. The best choice of product depends on the needs of the particular application and includes the following products:

Pharmacidal

Product Name	Catalogue No.	Unit Size	Storage Temp.
Pharmacidal, spray bottle for disinfecting surfaces	IC-110100	1 liter	AMB
	IC-110100-L	250ml	AMB
	IC-110100-B	100ml	AMB
Pharmacidal, for disinfecting surfaces (without sprayer)	IC-110100-G	5 liter	AMB

Disinfectant solution for incubators and sterile benches in cell culture and molecular biology laboratories.

The problem of contamination in incubators and/or sterile workbenches is often serious, leading to extensive damage. Pharmacidal solution prevents contamination, growth of fungi (and spores), bacteria (and spores) mycoplasma and viruses (including HIV and Hepatitis B).

The active ingredients are quaternary benzylammonium compounds, and the solution does not contain mercury, formaldehyde, phenol or alcohol.

Furthermore, Pharmacidal is non-toxic and biodegradable. It has also been found to be fully compatible with common work surfaces.

Recommended use

- Spray incubators once every 2 weeks.
- Spray sterile benches once a day, or preferably before each laboratory worker begins using the work area.
- The surfaces to be disinfected should be completely wet by spraying.
- Allow to dry.

Aquaguard-1

Product Name	Catalogue No.	Unit Size	Storage Temp.
AQUAGUARD-1 Solution for disinfecting water baths of CO ₂ incubators	01-867-1B	100ml	-20°C

100ml of 100X concentrated solution for disinfecting CO₂ water baths. (Use 50ml per 5 liters of water in bath).

The water required for humidity is a source of contamination that disperses in the incubator. In order to disinfect the water we recommend Aquaguard-1 Solution, which contains a disinfectant that does not cause damage to the stainless steel tray, is non-toxic, non-volatile, and extremely effective.

The water should be replaced with sterile water every two to four weeks, adding 50ml of Aquaguard-1 per 5 liters of water.

Preventive treatment as described above will prevent damage that can be caused as a result of contamination to the tissue culture. In addition, it will also prevent the necessity of dealing with contamination that has dispersed in the incubator and causes repeated contamination of the tissue culture.

Aquaguard-2

Product Name	Catalogue No.	Unit Size	Storage Temp.
AQUAGUARD-2 Solution for disinfecting ordinary water baths	01-916-1E	50ml	-20°C

50ml of 500X concentrated solution for prevention of microbial growth in water baths. (Use 2ml per 1 liter of water).

Aquaguard-2 is intended for disinfecting various kinds of water baths from bacteria and fungi. It is recommended to use 2ml of Aquaguard-2 for each liter of water in the bath, and to repeat the procedure every 1-2 weeks. After 4-6 weeks, the bath should be emptied and refilled with water containing Aquaguard-2.

Attachment Factors

10 Attachment Factors

Attachment factors are structural proteins or protein-like substances that have adherent capabilities and increase cell-substrate interactions in a culture dependent attachment milieu. A number of glycoproteins have been identified that promote and/or influence in vitro cell attachment to the surface or substratum of the culture vessel.

Normal attachment, growth and development of many cell types are dependent on attachment factors and extracellular matrix components. While some cells are able to synthesize these components, others require an exogenous source, particularly when grown in serum-free culture.

The growth and differentiation of anchorage-dependent cells are often strongly influenced by either glass or plastic culture flasks utilized as a substrate. In order to facilitate attachment, cell spreading, growth, morphology, differentiation, and motility of your cells, Biological Industries offers an extensive line of attachment and matrix factors. Each lot is cell culture tested to assess its ability to promote cell attachment and spreading.

Collagen is a major structural protein of extracellular matrix and is the principal protein found in connective tissues. It is found not only in the organic portion of bones, skin, teeth and tendons, but also occurs in other parts of the body as fibrous inclusions. Like other fibrous proteins, collagen is not readily available unless it undergoes heat treatment such as boiling which converts collagen into gelatin. It is an unusual protein, rich in amino acids such as glycine, lysine, proline and others but unfortunately not enough of the essential amino acids. Usually the gelatin derived from collagen is a relatively poor-quality protein.

Gelatin solution (0.1%) is intended for coating cell culture flasks or plates utilized in the growth of Mouse ES cells without a feeder layer. Leukemia Inhibitory Factor (LIF), a pleiotropic, polyfunctional glycoprotein (IL-6) cytokine, should be added to the medium. This impacts growth promotion and prevents cell differentiation on a wide array of various tissue types and target cells.

Human Fibronectin Solution, 1mg/ml

Product Name	Catalogue No.	Unit Size	Storage Temp.
Human Fibronectin Solution, 1mg/ml	05-750-1H	1ml	2-8°C
	05-750-1F	5ml	2-8°C

Human Fibronectin (hFN) was tested and found suitable matrix for many cell types as well as for stem cells (e.g. mesenchymal stem cells). Biological Industries' hFN is obtained by affinity purification on gelatine-sepharose from human plasma.

Features

- A complete, ready-to-use solution.
- Suitable for various animal cells.
- Performance tested.

Source

Human plasma.

Concentration

1mg/ml, based on E (1%, 280nm)=12.8.

Identification

A major single band of approx. 220,000 Dalton is evident.

Quality Control

This product has been tested for its ability to promote the attachment and spreading of BHK-21 cells in Serum-free medium.

Suggested Coating Procedures

The recommended concentration of the Fibronectin is 5 mg per ml of medium (or 2-10 $\mu\text{g}/\text{cm}^2$). The Fibronectin should be added to DPBS or growth medium in the growth vessel for at least 30 minutes in incubator (37°C). Before seeding, wash the vessel with DPBS or medium. When the medium is replaced in the days following initial seeding no further Fibronectin is required.

References

1. Ruoslahti E. and Ruoslahti. E. Int. J. cancer 20, 1-5 (1977).
2. Miekka S.I e., al. Thrombosis Research 27, 1-14 (1982).
3. Mosesson MW. and Umfleet RA. The J. of Biological Chemistry Vol.245, No.21 5728-5736 (1970)
4. Vuento M. and Vaheri A. Biochem. J. (1979) 183, 331-337.

Bovine Fibronectin Solution

Product Name	Catalogue No.	Unit Size	Storage Temp.
Bovine Fibronectin Solution 1mg/ml	03-090-1-01	1ml	2-8°C
	03-090-1-05	5ml	2-8°C

Fibronectin is an attachment factor that facilitates the attachment and cytoplasmic spreading of all types of anchorage-dependent cells. Fibronectin is particularly useful for the culture of cells that are not capable of synthesizing their own biomatrix or when culturing cells in serum-free medium.

Source

Irradiated citrated bovine plasma.

Description

A clear sterile solution containing Fibronectin, obtained by affinity purification on gelatin-sepharose from bovine plasma. The Fibronectin solution contains buffer salts.

Concentration

1mg/ml, based on E (1%, 280nm) =12.8

Identification

A major single band of approx. 220,000 Dalton is evident.

Suggested coating procedure

The Fibronectin should be added to the growth medium in the culture vessel which is then placed in an incubator 30-60 minutes before seeding. The recommended concentration of the Fibronectin is 5 micrograms per ml of medium. When the medium is replaced in the days following initial seeding, no further Fibronectin is required.

References

1. Ruoslahti E. Int. J. Cancer 20, 1-15 (1977).
2. Miekka S.I Et Al Thrombosis Research 27, 1-14 (1987).
3. Mosesson M.W The J. of Biological Chemistry Vol.245, No.21 5728-5736 (1970).

MSC Attachment Solution

Product Name	Catalogue No.	Unit Size	Storage Temp.
MSC Attachment Solution	05-752-1S	0.1ml	2-8°C
	05-752-1F	1ml	2-8°C
	05-752-1H	5ml	2-8°C

A xeno-free (XF) solution for facilitating attachment and spreading of hMSC in serum-free culture system.

Features

- Ready-to-use solution.
- Without xenogenic components (XF).
- Suitable for hMSCs from various sources.
- Designed for use in serum-free culture systems.

For more information see page 10-11

Collagen Type I, Rat Tail

Product Name	Catalogue No.	Unit Size	Storage Temp.
Collagen Type I, Rat Tail	01-990-100	100mg	2-8°C

Collagens are a family of highly characteristic fibrous protein found in all multicellular animals and are critical in cell adhesion. Collagen Type I is found in several tissues including skin, connective tissue cartilage and bone.

Collagen Type I is an attachment factor that facilitates the attachment and cytoplasmic spreading of all types of anchorage-dependent cells, when used as a thin layer on a tissue culture surface. As a gel, Collagen I enhances expression of cell-specific morphology and function.

Gelatin Solution 0.1%

Product Name	Catalogue No.	Unit Size	Storage Temp.
Gelatin Solution (0.1%)	01-944-1A	500ml	2-8°C
	01-944-1B	100ml	2-8°C

Qualified for Mouse Embryonic Stem (ES) Cells.

Mouse Embryonic Stem Cells (mESCs) are used to generate mouse mutants by gene targeting and blastocyst-mediated transgenesis. Undifferentiated ES cells may be maintained in vitro for extended periods without loss of differentiation capacity when re-implanted back into a blastocyst. Well-established general culture conditions usually require the undifferentiated ES cells to be grown on inactive feeder cell layers or on gelatin-coated plates with Leukemia Inhibitory Factor (LIF) in the culture medium to influence cell growth and function. Growth and differentiation of anchorage-dependent cells are strongly influenced by glass or plastic cultureware offered as a cell-substrate interactive platform. Cell growth rates may be exponentially improved by specialized surface treatments or coating with attachment factors such as Gelatin Solution with LIF.

Application

Used for the attachment of a variety of cell types.

Properties

Sterile, Endotoxin Tested and Cell Culture Tested.
Product is ready to use for plating.

NutriMatrix™ - ECM Coated Plastic Ware

Coated with extracellular matrix (ECM) simulates in vivo conditions.

One of the drawbacks in growing cells In Vitro using conventional tissue culture techniques is that the cells rest on plastic rather than on their natural biological support. This natural support is a complex network of macromolecules known as the extracellular matrix or ECM. ECM holds cells and tissues together and provides a highly organized lattice within which cells can migrate and interact with each other. The matrix plays an active and complex role in regulating the behavior of cells that are in contact with it, influencing their shape, migration, proliferation and metabolic functions. In contrast, cells grown on plastic lose many of their natural differentiated properties due to the lack of interaction with ECM.

ECM is composed of different types of collagen glycosaminoglycans, proteoglycans and glycoproteins^[1]. It resembles the vascular subendothelial basal lamina in its organization and macromolecular constituents (fibronectin, lamin, collagen types III, IV and V, and sulfated proteoglycans)^[2].

Advantages

Rapid attachment; high plating and cloning efficiencies; rapid proliferation, high saturation density; lower requirements for serum and added growth factors; better response to physiologically occurring hormones; expression of differentiated functions; longer life span for cells; flattening and morphological changes; and improved plating consistency.

Among the cells types showing a favorable response to ECM are human, bovine and other origin.

Research Applications

- **Epithelial Cells:**
NutriMatrix™ ECM-coated plastic vessels with serum-free medium enable a higher rate of success in growing normal and malignant human epithelial cells from biopsy specimens. ECM induces changes in cell shape not observed in cells grown on plastic or isolated components of the ECM. Cells which for different reasons do not flatten or spread on plastic do so rapidly on ECM.
- **Hormone Secretion Research:**
NutriMatrix™ ECM-coated plastic vessels with serum-free media support the maintenance and normal function of hormone secreting cells such as pancreatic islet cells, hepatocytes pituitary cells, granulosa cells, etc.
- **Secretion of Cellular Products:**
The ECM/serum-free medium combination promotes research possibilities on various cellular products.

Hormone Response Research:

ECM effects cell shape and hormone responsiveness. As expected the cells do not respond when maintained on artificial substrata or isolated components of the ECM.

Biotechnology Applications⁽³⁾

• Yield and Differentiation:

The maintenance and growth of differentiated cells on ECM is expected to promote a high yield of various hormones and growth factors in tissue culture.

• Purification:

Growth of cells in serum-free media will facilitate the purification of various cellular products that are secreted into the medium. Purification will be relatively simple due to the absence of serum proteins.

• Production:

Large-scale growth of cells on ECM can be performed in bulk cell culture NutriMatrix™ vessels coated with ECM, or on NutriMatrix™ ECM-coated microcarriers. Using these techniques, continuous rather than batch processes can be developed.

• Growth Factor Secretion:

Growth factors may be produced in better yields by human cells cultured on ECM rather than on plastic and can then be purified and used for research and clinical applications.

• In Vitro Toxicological Testing And Drug Screening:

The growth of cells on ECM in serum free medium may reduce the cost and simplify the procedure of studying the effect on cells of single drugs, drug combinations and hormones or where a single component is being tested at a time.

• Neurobiology:

ECM has been shown to support the attachment and maintenance of neurons from various sources and to promote the outgrowth and directed elongation of neurites.

Item	Packaging (unit/Pack)	Catalogue No.
Tissue Culture Dishes 35 mm	5	E-TCP-35
Tissue Culture Dishes 60 mm	5	E-TCP-60
Tissue Culture Dishes 90 mm	5	E-TCP-90
Tissue Culture Flasks 25 cm ₂	5	E-TCF-25
Tissue Culture Flasks 80 cm ₂	5	E-TCF-80
Microtiter 96-Well Plate	1	E-TCMT-F
4-Well Culture Plate	1	E-TCMW-4
6-Well Culture Plate	1	E-TCMW-6
12-Well Culture Plate	1	E-TCMW-12
24-Well Culture Plate	1	E-TCMW-24
Coverslips (Round, 22 mm)	5	E-TCCS-P22
Four 13mm Coverslips In 4-Well Plate	1	E-TC-IF-13
Eight 12mm Filters in 24- Well Plate	1	E-TC-M-12
Eight Well Lab-Tek Chamber Slide	1	E-LT-8

Storage

NutriMatrix™ ECM-coated plastic vessels are shipped at ambient temperature and should be stored at 2-8°C upon arrival.

⁽¹⁾ Cancer research 46: 3653 (1986).

⁽²⁾ Blood 65: 1477 (1985).

⁽³⁾ Metal Ions in Biology and Medicine. Volume 4 by Philippe Collery. Published by John Libbey Eurotext, 1996.

Cell Dissociation

Cell Dissociation

11

11 Cell Dissociation

Most cell cultures grow as a single thickness cell layer or sheet attached to a substrate known as a monolayer. When subculturing adherent cells, these intercellular and cell-to-substrate links or connections must be gently dissociated. Proteolytic enzymes, such as trypsin (i.e. a serine peptidase), breaks or gently separates these bonds by creating a single-cell suspension from which new subcultures are realized. Trypsin solutions are widely utilized as cell dissociation reagents for continuous cell culture of adherent growing cells. Trypsin proteolysis or trypsinization is a process in which proteins have been digested or treated with trypsin and are thus said to be trypsinized. Biological Industries' Trypsin is designed to gently dissociate cells from almost any support substrates, as well as from each other, in order to actualize cell manipulation techniques, and for other studies that require intact cell-surface proteins. Trypsin is available in a varied array of formulations with or without EDTA. EDTA is a chelator that binds calcium and magnesium ions that may otherwise inhibit the trypsin activity, which then hydrolyzes and gains access to the intercellular bonds (cell-cell and/or cell-substrate bonds).

Recombinant Trypsin Solutions

Product Name	Catalogue No.	Unit Size	Storage Temp.
Recombinant Trypsin Solution	03-078-1B	100ml	-20°C
	03-078-1A	500ml	-20°C
Recombinant Trypsin-EDTA Solution	03-079-1B	100ml	-20°C
	03-079-1A	500ml	-20°C

An animal components-free cell dissociation solution, designed as an alternative to porcine trypsin for the dissociation of cells. Special, animal components-free (ACF) recombinant trypsin solutions, developed as an alternative to porcine trypsin. The solutions do not contain any chymotrypsin, carboxypeptidase A, and other protease contaminant.

Recombinant Trypsin Solution formulations were developed for efficient dissociation of adherent cell types from surfaces and tissues and were optimized for sensitive cells, such as primary human mesenchymal stem cells (hMSC). The addition of EDTA usually accelerates the dissociation phase.

Recombinant Trypsin Solutions are pure enzyme solutions, which help maximize the yield of functionally viable cells from culture vessels, while preventing the toxicity effect induced by other non desirable proteases. In addition, recombinant trypsin eliminates the risk of viruses, or other potential adventitious agents found in animal derived components.

Source

Recombinant Trypsin Solutions are produced by submerged microbial fermentation. They are derived from a production process which does not utilize any raw materials and/or processing aids of animal origin.

Features

- Ready-to-use.
- Animal component-free.
- Increased specificity.
- Eliminates contaminating activities found in bulk production of enzymes.
- Free from undesirable proteases such as carboxypeptidase A and chymotrypsin.
- Optimized for hMSCs (from variety of sources), cultured in both serum-free and serum-containing systems.

Crystalline Trypsin Solution & Soybean Trypsin Inhibitor Solution

Product Name	Catalogue No.	Unit Size	Storage Temp.
Crystalline Trypsin Solution (0.02%) Without Phenol Red	03-047-1A	500ml	-20°C
	03-047-1B	100ml	-20°C
Soybean Trypsin Inhibitor 50X Conc., 5mg/ml	03-048-1C	20ml	-20°C

Crude trypsin is often the subculturing agent of choice for cell dissociation/ disaggregation of adherent cells, although the treatment may be cytotoxic if prolonged. Over-trypsinization is a common cause of subculture problems.

Regarding the use of crude trypsin, some important facts must be noted:

- Cells must **NEVER** remain in the crude trypsin for longer than 3-5 minutes as they may be seriously damaged in the process (i.e. damage to the intracellular proteins).
- Cells should **NEVER** be left without a fluid layer.

The use of crystalline trypsin, rather than crude trypsin, most often performs better long-term cell growth in serum-free medium formulations. It is specifically formulated to have a gentle nature with much better cell viability, in which the cells are not subject to the vagaries of time and circumstance as when the cruder forms of trypsin are utilized.

Some of the advantages of crystalline trypsin versus the cruder trypsin forms:

1. Crystalline trypsin does not damage cells after prolonged exposure.
2. Crystalline trypsin does not require multiple-change procedures and thus is less labor-intensive.
3. Crystalline trypsin maintains better cell viability and enhances the process of cell passaging.

4. Crystalline trypsin is not as cytotoxic to cells with all the negative ramifications of crude trypsin.
5. Biological Industries' Crystalline Trypsin Solution also contains additives that protect the cell wall, enhancing cell viability.

In a serum-free culture environment, the cells must be separated by rapid centrifugation or by utilizing trypsin inhibitors such as Soybean Trypsin Inhibitor (SBTI). SBTI is a single polypeptide that forms a stable, stoichiometric, enzymically inactive complex with trypsin, thereby reducing the availability of trypsin by somewhat binding chymotrypsin. With Biological Industries' Soybean Trypsin Inhibitor Solution, any excess Crystalline Trypsin Solution may be completely neutralized, thereby avoiding the use of serum for this purpose. The cells may then be re-suspended successfully in a suitable growth medium.

The use of animal-derived components in Biopharmaceutical Manufacturing is experiencing ever-increasing regulatory scrutiny. Therefore, there is the need to develop non-animal source products for cell culture. Trypsin is an essential product for cell culture manipulation. However, it is purified from animal-source materials with one unfortunate notable disadvantage: contamination from variegated sources such as viruses, other potential adventitious agents and other unwanted enzymes.

Non-Enzymatic Cell Dissociation Solution

Product Name	Catalogue No.	Unit Size	Storage Temp.
Non-Enzymatic Cell Dissociation Solution	03-071-1B	100ml	2-8°C

Cell Dissociation Solution is a special, non-enzymatic formulation with a proprietary mixture of chelators for gently dislodging adherent cell types from culture vessels. Cell Dissociation Solution helps to maximize the yield of functionally viable cells from these culture vessels. It is a non-enzymatic, protein-free and animal-component free solution. Another major advantage is that cells can be exposed to this solution for longer periods of time without the risk of subjecting them to protein digestive enzymes such as trypsin. However, the solution is not recommended for cells with very adhesive properties. For those cell lines which are difficult to dislodge, Biological Industries has developed a Papain Dissociation Solution.

Features

Contains a proprietary mixture of chelators. Contains no enzymes or proteases.

- Works with serum-free and serum-containing media.
- Reduces the risk of cell damage associated with trypsin.
- Chemically defined.
- Contains no products of animal origin.
- Supplied as a ready-to-use solution.

Papain Dissociation Solution

Product Name	Catalogue No.	Unit Size	Storage Temp.
Papain Dissociation Solution	03-072-1B	100ml	-20°C

Papain is a nonspecific, endolytic, sulfhydryl protease or protein-cleaving enzyme, known as cysteine-endopeptidase, and is derived and isolated from papaya fruit (i.e. *Carica papaya*). More specifically, it is isolated from the papaya latex, which is then utilized in a wide variety of applications. Papain is commonly used in cell isolation procedures, where it has proven to be more efficient and less destructive than other proteases on certain tissues such as and including, among others, the dissociation of retinal neurons⁽¹⁾, in the preparation of primary neurons from the visual cortex of postnatal rats⁽²⁾, and for the isolation of smooth muscle cells⁽³⁾.

Papain has a wide specificity in that it will degrade most protein substrates more extensively than the pancreatic proteases and has been proven not only to manifest fewer untoward and negative ramifications producing less cell and tissue trauma, but also to be much more effective than other available proteases. Biological Industries' Papain Dissociation Solution is a ready-to-use solution and is one of our non-animal alternatives for trypsin.

Physical Properties and Kinetics

Papain is a cysteine protease hydrolase enzyme of the peptidase C1 family derived from the papaya family, *Carica papaya* and the mountain papaya, *Vasconcellea cundinamarcensis*. It consists of a single peptide chain with three disulfide bridges and a sulfhydryl group necessary for the activity of the enzyme.

Specificity

Papain is more effective in digesting most protein substrates more extensively and effectively than pancreatic proteases. It further exhibits broad specificity cleaving peptide bonds of such basic amino acids as leucine and glycine. In addition to the aforementioned activity, it also hydrolyzes esters and amides.

⁽¹⁾ Shen J., et al., Japanese Journal of Physiology, 1995.

⁽²⁾ Huettner, J.E. Baughman, R.W., Journal Of Neuroscience, 1986.

⁽³⁾ Kinoshita, K. et.al., American Journal of Physiology, Gastrointestinal and Liver Physiology, 2003 and Driska, S.P. et.al., Journal of Applied Physiology, 1999.

Ordering Information

Product Name	Trypsin Conc.	Catalogue No.	Unit Size	Storage Temp.
Trypsin Solution A With Calcium and Magnesium Without Phenol Red	0.25%	03-045-1B	100ml	2-8°C
Trypsin Solution B Without Calcium and Magnesium Without Phenol Red	0.25%	03-046-1A 03-046-1B	500ml 100ml	-20°C -20°C
Trypsin Solution B Without Calcium and Magnesium Without Phenol Red, 10x Concentrate	2.50%	03-046-5A 03-046-5B	500ml 100ml	-20°C -20°C
Crystalline Trypsin Solution Without Phenol Red	0.02%	03-047-1A 03-047-1B	500ml 100ml	-20°C -20°C
Soybean Trypsin Inhibitor 50x Conc., 5mg/ml		03-048-1C	20ml	-20°C
Trypsin EDTA Solution A, EDTA (0.02%) With Phenol Red	0.25%	03-050-1A 03-050-1B	500ml 100ml	-20°C -20°C
Trypsin EDTA, EDTA 0.2% , 10X Conc. ¹	0.50%	03-051-5B 03-051-5C	100ml 20ml	-20°C -20°C
Trypsin EDTA Solution B, EDTA (0.05%) With Phenol Red	0.25%	03-052-1A 03-052-1B	500ml 100ml	-20°C -20°C
Trypsin EDTA Solution C, EDTA (0.02%) With Phenol Red	0.05%	03-053-1A 03-053-1B	500ml 100ml	-20°C -20°C
Trypsin EDTA Solution C, EDTA (0.02%) Without Phenol Red	0.05%	03-054-1A 03-054-1B	500ml 100ml	-20°C -20°C
Non-Enzymatic Cell Dissociation Solution		03-071-1B	100ml	2-8°C
Papain Dissociation Solution		03-072-1B	100ml	-20°C
Recombinant Trypsin Solution		03-078-1B 03-078-1A	100ml 500ml	-20°C -20°C
Recombinant Trypsin-EDTA Solution		03-079-1B 03-079-1A	100ml 500ml	-20°C -20°C

⁽¹⁾ See Chapter 3
See formulations on page 106

Antibiotics

12 Antibiotics

Antibiotics are natural substances of bacterial origin derived entirely or partially from certain microorganisms that are used to treat bacterial or fungal infections by selective inhibition. Chemotherapeutic agents refer to any synthetic or man-made substance that actually characterizes the so-called newer antibiotics today that are essentially chemically-modified or chemically synthesized biological products. Today, the term 'antibiotic' is used to refer to all types of antimicrobial agents. The distinctions between both natural and man-made synthetic substances are designed, in one way or another, to block one or several crucial metabolic pathways without untoward manifestations to the host, or in this case, the cell culture. Preventing cell culture contamination is an essential part of all animal cell culture. The risk of contamination may be eliminated by effective aseptic/sterile techniques and the judicious use of antibiotics.

Antibiotics may be classified into several key groups by virtue of their mechanism of action which include:

- Inhibition of Cell-Wall Synthesis.
- Inhibition of Nucleic Acid Synthesis (i.e. RNA/DNA).
- Inhibition of Protein Synthesis.
- Inhibition or Interference of Microtubule Function.

The major advantage of some antibiotics is their ability to selectively target crucial and specific cell processes which either kill the microorganism in question or prevent them from reproducing unabated. Antibiotics are often also categorized by:

- Their Spectrum of Activity.
- Their Bacteriostatic/Bactericidal Properties.
- Their Gram-Negative or Gram-Positive Characteristics.

Antibiotics are ineffective against viruses.

Although many laboratories use antibiotics on a regular basis, the decision to use them to prevent cell culture contamination must be based on the individual researcher's requirements and experience.

The appropriate antibiotics may be added to culture media to eliminate microbial contaminants. The most commonly encountered microorganisms are bacteria, yeast, other fungi and mycoplasma while the most common routes of contamination are poor aseptic technique and use of non-sterile medium components.

Biological Industries offers a wide range of effective antibiotics that include solutions, mixtures, and powdered chemical formulations. The following table is presented as a general guide for use in cell culture.

Product Name	Catalogue No.	Unit Size	Storage Temp.
Amphotericin B⁽²⁾ Solution 250 microgram/ml	03-028-1B	100ml	-20°C
	03-028-1C	20ml	-20°C
Amphotericin B⁽²⁾ Solution 2500 microgram/ml	03-029-1B	100ml	-20°C
	03-029-1C	20ml	-20°C
Nystatin⁽¹⁾ Suspension 10,000 units/ml	03-030-1C	20ml	-20°C
Penicillin-Streptomycin Solution Penicillin G Sodium Salt, 10,000 units/ml Streptomycin Sulfate, 10mg/ml	03-031-1B	100ml	-20°C
	03-031-1C	20ml	-20°C
Penicillin-Streptomycin Solution, 10X Conc. Penicillin G Sodium Salt, 100,000 units/ml Streptomycin Sulfate, 100mg/ml	03-031-5B	100ml	-20°C
	03-031-5C	20ml	-20°C
Penicillin-Streptomycin Nystatin⁽¹⁾ Solution Penicillin G Sodium Salt, 10,000 units/ml Streptomycin Sulfate, 10mg/ml Nystatin ⁽¹⁾ , 1,250 units/ml	03-032-1B	100ml	-20°C
	03-032-1C	20ml	-20°C
Penicillin-Streptomycin Amphotericin B⁽²⁾ Solution Penicillin G Sodium Salt, 10,000 units/ml Streptomycin Sulfate, 10mg/ml Amphotericin B ⁽²⁾ , 25 microgram/ml	03-033-1B	100ml	-20°C
	03-033-1C	20ml	-20°C
Penicillin-Streptomycin Neomycin Solution Penicillin G Sodium Salt, 10,000 units/ml Streptomycin Sulfate, 10mg/ml Neomycin Sulfate, 10mg/ml	03-034-1B	100ml	-20°C
	03-034-1C	20ml	-20°C
Gentamicin Sulfate Solution 50 mg/ml	03-035-1B	100ml	AMB
	03-035-1C	20ml	AMB
Kanamycin Sulfate Solution 10mg/ml	03-049-1B	100ml	-20°C
	03-049-1C	20ml	-20°C

⁽¹⁾ **Nystatin** is the generic name for **Mycostatin**[®] which is the registered trade mark of E.R. Squibb & Sons.

⁽²⁾ **Amphotericin B** is the generic name for **Fungizone**[®] which is the registered trade mark of E.R. Squibb & Sons.

Use of Antibiotics in Mammalian Cell Culture

Antibiotics are secondary metabolites which are produced by certain strains of bacteria and fungi. In cell culture, antibiotics have long been used to prevent the growth of contaminating bacteria and fungi. The following table is provided as a guide for antibiotics selection and appropriate concentrations. Refer to pharmacology guides for antibiotic incompatibilities and other properties not included in the table.

Product Name	Catalogue No.	Conc.	Storage Temp.	Mode of Action	Suggested Working Conc.
Amphotericin B	03-028-1	250µg/ml	-20°C	Inhibition of cell membrane permeability (fungi and yeasts)	1-10ml/lit
Amphotericin B	03-029-1	2,500µg/ml	-20°C	Inhibition of cell membrane permeability (fungi and yeasts)	0.1-1ml/lit
Nystatin ⁽¹⁾	03-030-1	10,000un/ml	-20°C	Inhibition of cell membrane permeability (fungi and yeasts)	1-10ml/lit
Penicillin-Streptomycin	03-031-1	Penicillin: 10,000un/ml Streptomycin: 10mg/ml	-20°C	Penicillin: Inhibition of cell wall synthesis Streptomycin: Inhibition of protein synthesis by binding to 30S subunit of the bacterial ribosome	10ml/lit
Penicillin-Streptomycin 10x	03-031-5	Penicillin: 100,000un/ml Streptomycin: 100mg/ml	-20°C	See: Penicillin-Streptomycin	1ml/lit
Penicillin-Streptomycin-Nystatin	03-032-1	Penicillin: 10,000un/ml Streptomycin: 10mg/ml Nystatin: 1,250un/ml	-20°C	See: Penicillin, Streptomycin and Nystatin	10ml/lit
Penicillin-Streptomycin-Amphotericin B	03-033-1	Penicillin: 10,000un/ml Streptomycin: 10mg/ml Ampho. B: 25µg/ml	-20°C	See: Penicillin, Streptomycin and Amphotericin B	10ml/lit
Penicillin-Streptomycin-Neomycin	03-034-1	Penicillin: 10,000un/ml Streptomycin: 10mg/ml Neomycin: 10mg/ml	-20°C	See: Penicillin and Streptomycin Neomycin: Inhibition of protein synthesis by binding to 30S subunit of the bacterial ribosome	10ml/lit
Gentamicin sulfate	03-035-1	50mg/ml	15-30°C	Inhibition of protein synthesis by binding to 30S subunit of the bacterial ribosome	1ml/lit
Kanamycin sulfate	03-049-1	10mg/ml	-20°C	Inhibition of protein synthesis by binding to 30S subunit of the bacterial ribosome	10ml/lit

⁽¹⁾ Suspension in water

Cell Viability

13 Cell Viability

Cell Proliferation Kit (XTT Based)

Product Name	Catalogue No.	Unit Size	Storage Temp.
Cell Proliferation Kit (XTT based)	20-300-1000	1000 assays	-20°C

Cell proliferation assays are widely used in cell biology for the study of growth factors, cytokines and media components, for the screening of cytotoxic agents and for lymphocyte activation.

The need for a reliable, sensitive and quantitative assay that would enable analysis of a large number of samples led to the development of methods, such as:

- Use of radioactive thymidine to label DNA in live cells.
- Use of Brdu to label DNA in live cells
(as a substitute for radioactive thymidine).

The above methods have a number of disadvantages, including: use of radioactive materials and relatively complex techniques. The use of tetrazolium salts, such as MTT, is based on the fact that live cells reduce tetrazolium salts into colored formazan compounds. The biochemical procedure is based on the activity of mitochondria enzymes which are inactivated shortly after cell death. This method was found to be very efficient in assessing the viability of cells.

A colorimetric method based on the tetrazolium salt, XTT, was first described by P.A. Scudiero in 1988. Whilst the use of MTT produced a non-soluble formazan compound which necessitated dissolving the dye in order to measure it, the use of XTT produces a soluble dye. The use of XTT greatly simplifies the procedure of measuring proliferation, and is, therefore, an excellent solution to the quantitating of cells and their viability without using radioactive isotopes. This kit was developed to assay cell proliferation in reaction to different growth factors, cytokines and nutrient components. In addition, it is suitable for assaying cytotoxicity of materials such as TNF or other growth inhibitors. XTT can be used as a non-radioactive substitute for cytotoxic tests based on the release of ⁵¹Cr from cells with no less sensitivity.

Advantages

- **Easy-to-use:** there is no requirement for additional reagents and/or the cell washing procedures.
- **Speed:** multiwell plates and an ELISA reader can be used for reading.
- **Sensitivity:** can be assayed even in low cell concentrations.
- **Accuracy:** dye absorbance is proportional to the number of cells in each well.
- **Safety:** there is no need for radioactive isotopes.

Kit Components

- XTT Reagent (10x5ml), a sterile solution containing the XTT reagent. The solution should be stored frozen and should not be exposed to light. To avoid repeated re-freezing, dividing the solution into a number of vials after defrosting the original vial is recommended.
Note: if sediment is present in the solution, heat the solution to 37°C and swirl gently until a clear solution is obtained.
- Activation Reagent (2x0.5ml), a sterile solution containing PMS (N-methyl dibenzopyrazine methyl sulfate). The solution should be stored frozen and should not be exposed to light. To avoid repeated re-freezing, dividing the solution into a number of vials after defrosting the original vial is recommended.
Note: if sediment is present in the solution, heat the solution to 37°C and swirl gently until a clear solution is obtained.

Assay Principles

The assay is based on the ability of metabolic active cells to reduce the tetrazolium salt XTT to orange colored compounds of formazan. The dye formed is water soluble and the dye intensity can be read at a given wavelength with a spectrophotometer. The intensity of the dye is proportional to the number of metabolic active cells. The use of multiwell plates and an ELISA reader enables testing a large number of samples and obtaining easy and rapid results. The test procedure includes cultivation of cells in a 96-well plate, adding the XTT reagent and incubation for 2-24 hours. During incubation an orange color is formed, the intensity of which can be measured with a spectrophotometer, in this instance with an ELISA reader. The greater the number of active cells in the well, the greater the activity of mitochondria enzymes, and the higher the concentration of the dye formed, which can then be measured and quantitated.

Typical Experiment:

The Cytotoxicity of Butylated Hydroxyanisole (BHA)

Butylated Hydroxyanisole (BHA)- synthetic antioxidant used in the food and cosmetic industry.

Mechanism of cytotoxicity

Low doses of BHA exerted a significant cytotoxic effect, associated with loss of mitochondrial function. As the concentration of BHA increases, morphological alterations in critical sub-cellular targets such as lysosomes, mitochondria and actin cytoskeleton, are observed. In parallel, BHA induced an irreversible loss of cell proliferative capacity, preceding apoptosis induction.

The cytotoxic system

Vero cells were exposed to increased concentrations of BHA (0-500µM) for 24 hours to create a cytotoxic system.

BHA cytotoxicity of Vero cells

Vero cells were cultured (5000 cells per well) in a 96 well plates for 24 hours. Cells were exposed to increased concentrations of BHA (0-500µM) for 24 hours, then viability was measured, using a colorimetric method (XTT Based Cell Proliferation Kit , Cat. No. 20-300-1000). XTT reagent was added and absorbance was measured (wavelength of 450nm and reference of 690nm) after a further 5 hours of incubation.

Figure 1: Determination of the cytotoxicity activity of BHA on vero cells.

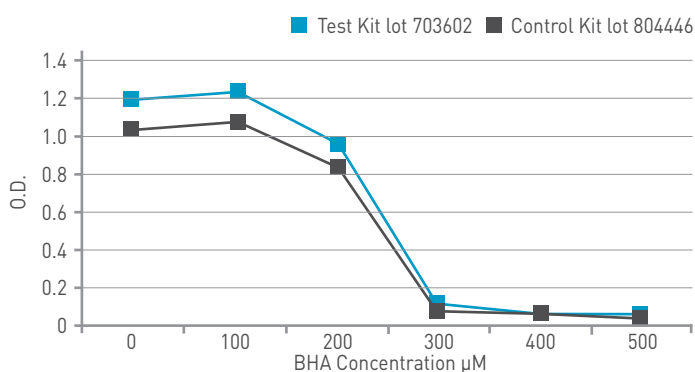
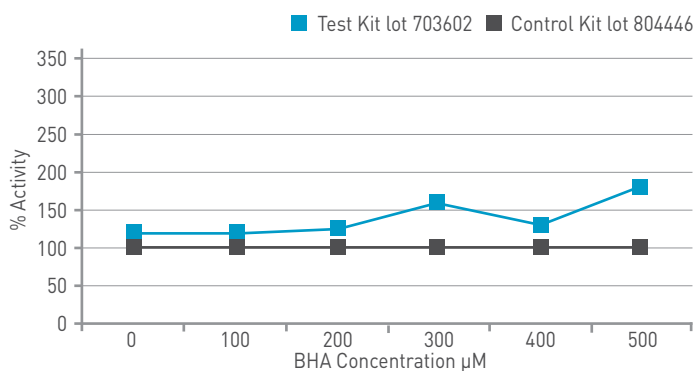


Figure 2: % Activity using test and control kit.

Each point of the control kit was defined as 100% activity. The % activity of the test kit is presented as percentage from the control kit at the same point.



Trypan Blue (0.5% Solution)

Product Name	Catalogue No.	Unit Size	Storage Temp.
Trypan Blue Solution, 5mg/ml in Saline	03-102-1B	100ml	AMB

Trypan Blue is the stain most commonly used to distinguish viable from nonviable cells. Viable cells exclude the dye, while nonviable cells absorb the dye and appear blue. Cells should be in suspension as single cells in buffered saline before counting.

Trypan Blue has a higher affinity for serum protein than for cellular proteins, so suspending cells in medium containing serum will generate a dark background.

Molecular Biology

14 Molecular Biology

EZ-RNA Total RNA Isolation Kit

Product Name	Catalogue No.	Unit Size	Storage Temp.
EZ-RNA Total RNA Isolation Kit	20-400-100	100ml	2-8°C

EZ-RNA is a complete kit with ready-to-use reagents for the isolation of total RNA from samples of human, animal, plant, yeast, bacterial and viral origin. EZ-RNA is based on disruption of cells in guanidinium thiocyanate/detergent solution, followed by organic extraction and alcohol precipitation of the RNA, which allows simultaneous processing of a large number of samples. The resulting RNA is suitable for the isolation of Poly A+ RNA or for Northern Blotting, Dot Blotting, or other analytical procedures. DNA and proteins can also be recovered from the interphase and the organic phase of the same sample.

Kit reagents

- Denaturing Solution, 50ml, contains: Guanidine Thiocyanate.
- Extraction and Phase Separation Solution, 50ml, contains: phenol and chloroform.

EZ-RNA II Total RNA Isolation Kit

Product Name	Catalogue No.	Unit Size	Storage Temp.
EZ-RNA II Total RNA Isolation Kit Without Chloroform, With BCP	20-410-100	100ml	2-8°C

With BCP instead of chloroform

EZ-RNA is a complete kit with ready-to-use reagents for the isolation of total RNA from samples of human, animal, plant, yeast, bacterial and viral origin. EZ-RNA II is based on disruption of cells in guanidine thiocyanate/detergent solution, followed by organic extraction and alcohol precipitation of the RNA, which allows simultaneous processing of a large number of samples. 1-Bromo-3-chloropropane (BCP) replaces chloroform, a highly volatile and toxic reagent used in molecular biology. Substituting BCP for chloroform in the EZ-RNA II kit reduces toxic material handling without any adverse effects on the quality of isolated RNA, DNA or proteins. The resulting RNA is suitable for the isolation of Poly A+ RNA or for Northern blotting, dot blotting, in vitro translation, molecular cloning, RT-PCR and RNase protection assays, or other analytical procedures. DNA and proteins can also be recovered from the interphase and the organic phase of the same sample.

Kit reagents

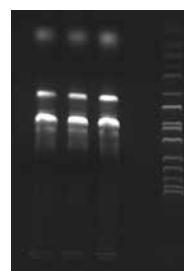
- Denaturing Solution, 50ml, contains: Guanidine Thiocyanate.
- Water-saturated phenol, 40ml.
- 1-Bromo-3-chloropropane (BCP), 9ml.

Assessing yield of total RNA

The yield of total RNA will vary depending on the tissue or cells from which it is obtained.

Tissue/sample Type	Amount of Starting Material	Yield of Total RNA
Rat liver	1mg	6µg
Rat skeletal muscle	1mg	0.9µg
Mouse brain	1mg	1.25µg
Mouse spleen	1mg	2.5µg
Mouse testes	1mg	2.5µg
Mouse thymus	1mg	0.85µg
Human cerebellum	1mg	0.8µg
Human prostate tumor	1mg	1µg
MCF-7 cell line	10 ⁸ cells	720µg
U251 cell line	10 ⁸ cells	950µg
Kidney	1mg	3µg
Placenta	1mg	1-4µg
Epithelial cells	10 ⁶ cells	8-15µg
Fibroblast cells	10 ⁶ cells	5-7µg
Plant poinsettia	1mg	0.7µg
Tobacco	1mg	0.8µg
Yeast	10 ⁷ cells	1-5µg
Bacteria	10 ⁹ cells	3-5µg

Figure 1: Total RNA extracted from hybridoma cells



RNA Save

Product Name	Catalogue No.	Unit Size
RNA Save	01-891-1A	500ml
	01-891-1B	100ml
	01-891-1C	20ml

Tissue storage solution for RNA stabilization

RNA Save is an aqueous, non toxic, tissue and cells storage solution intended for the preservation of RNA for later isolation. Samples in RNA Save solution can be stored indefinitely at -20°C or -80°C with no RNA degradation. RNA save solution can be used for the storage of tissues, cells, bacteria and yeasts. The solution may not be effective for the storage of waxy plant tissue and bone because of poor penetration of the solution.

RNA Save is compatible with most RNA isolation methods.

Storage

RNA Save should be stored at room temperature. If precipitation is seen, warm the solution to 37°C and mix carefully for resolubilization.

RNase-ExitusPlus™

Product Name	Catalogue No.	Unit Size
RNase-ExitusPlus™	01-897-1L	250ml
	01-897-1B	100ml

A solution for the decontamination of RNase

RNase-ExitusPlus™ is a non-alkaline, non-corrosive and non-carcinogenic cleansing solution that is highly active against RNase contamination. RNase-ExitusPlus™ has been demonstrated to inactivate more than 20µg of RNase A dried onto the bottom of a microcentrifuge tube. RNase-ExitusPlus™ is heat resistant.

Features

These are the new and unique characteristics of RNase-ExitusPlus™:

- Catalytic and cooperative effects of the components cause a very rapid nonenzymatic, non-sequence-specific degradation of protein and RNase molecules.
- All components of RNase-ExitusPlus™ are readily biologically degradable and not harmful or toxic for humans.
- No aggressive mineral acids or alkaline substances are used. Equipment and materials are not damaged or corroded even after prolonged incubation time.

- No organic solvents or volatile components, no toxic fumes.
- Elevated temperatures above approx. 50°C speed up the reaction and the efficiency/activity!

RNase-ExitusPlus™ is ready-to-use for eliminating RNase from any surface including the interior of microcentrifuge tubes. By following a few simple decontamination instructions, RNase is completely inactivated and removed.

Storage

RNase-ExitusPlus™ should be stored at room temperature; at colder temperatures a precipitate may form which is easily brought into solution at 37°C.

EZ-DNA Genomic DNA Isolation Kit

Product Name	Catalogue No.	Unit Size
EZ-DNA Genomic DNA Isolation Kit	20-600-50	50ml

EZ-DNA is a non-organic and ready to use reagent for the isolation of genomic DNA from samples of human, animal, plant, yeast, bacterial and viral origin. EZ-DNA is based on disruption of cells in guanidine-detergent lysing solution that hydrolyzes RNA and allows the selective precipitation of DNA from a cell lysate with ethanol. Following an ethanol wash, DNA is solubilized in water or 8 mM NaOH. There is no phenol in EZ-DNA. The protocol is fast and permits isolation of genomic DNA from a large number of samples of small or large volumes. The procedure can be completed in 10-30 minutes with DNA recovery of 70-100%. The isolated DNA can be used, without additional purification, for southern analysis, dot blot hybridization, molecular cloning, RFLP, PCR and other molecular biology and biotechnology applications.

Kit reagents

50ml solution containing Guanidinium Isothiocyanate.

Storage

EZ-DNA should be stored at room temperature. Storing at lower temperatures will cause the Guanidine Isothiocyanate to come out of the solution. If the reagent is warmed, the Guanidine Isothiocyanate should resolubilize instantly.

Assessing yield of genomic DNA

The yield of genomic DNA will vary depending on the tissue or cells from which it is obtained.

Tissue/sample Type	Amount of Starting Material	Yield of Total RNA
Liver	1mg	3-4µg
Kidney	1mg	3-4µg
Skeletal muscle	1mg	2-3µg
Brain	1mg	2-3µg
Placenta	1mg	2-3µg
Human cells	10 ⁶ cells	5-7µg
Rat cells	10 ⁶ cells	5-7µg
Mouse cells	10 ⁶ cells	5-7µg
Lung	1mg	3-5µg
Heart	1mg	2-3µg
Plant leaf	1gr	20-200µg
Whole blood	1ml	20-40µg
Sf9 cells	10 ⁷ cells	170-180µg
E.coli cells	10 ⁹ cells	30-40µg
Mouse tail	1mg	0.4-3µg

RBC Lysis Solution

Product Name	Catalogue No.	Unit Size	Storage Temp.
RBC Lysis Solution (for use with EZ-RNA and EZ-DNA Kits for whole blood)	01-888-1B	100ml	2-8°C

Red Blood Cells (RBC) Lysis Solution is intended for use in isolation of leukocytes from whole blood. RBC Lysis Solution selectively lyses the erythrocytes leaving the leukocytes. The resulting white blood cells can be readily lysed and processed when isolating nucleic acid with EZ-RNA, EZ-DNA or any other method for nucleic acid isolation from whole blood.

EZ-Plant

Product Name	Catalogue No.	Unit Size	Storage Temp.
EZ Plant (for use with 20-600-50)	01-893-1D	10ml	2-8°C

For use with EZ-DNA Kit to assist with DNA isolation from plant tissues containing polyphenolics and polysaccharides.

The EZ-Plant solution contains a high molecular weight polymer, Polyvinylpyrrolidone (PVP), which binds the reactive polyphenolic and polysaccharide contaminants present in plant tissues.

EZ-Blood

Product Name	Catalogue No.	Unit Size	Storage Temp.
EZ Blood (for use with 20-600-50)	01-894-1B	100ml	2-8°C

For use with EZ-DNA kit to assist with DNA isolation from frozen or fresh blood samples.

EZ Blood buffer solution is intended for the isolation of genomic DNA from frozen or fresh blood. The procedure includes a nuclear isolation step prior to DNA extraction. Work time required is 10 minutes.

EZ-ECL Enhanced Chemiluminescence Detection Kit for HRP

Product Name	Catalogue No.	Unit Size	Storage Temp.
EZ-ECL Kit	20-500-120	1200cm ² (120ml)	2-8°C

Chemiluminescence Detection kit for HRP is a complete kit with ready-to-use reagents for Enhanced chemiluminescent detection of immobilized proteins (Western blotting) or immobilized nucleic acids (Southern or Northern blotting), conjugated with HRP directly or indirectly. The use of enhanced chemiluminescence was introduced by Thorpe and Kricka. In the presence of hydrogen peroxide (H₂O₂), Horseradish peroxidase (HRP) catalyzes the oxidation of cyclic diacylhydrazides, such as luminol.

Immediately following the oxidation, the luminol is in an excited state (intermediate reaction product), which decays to the ground state by emitting light. Strong enhancement of the light emission is produced by enhancers, such as phenolic compounds. Using this method, it is possible to detect membrane immobilized specific antigens, or sequences of nucleic acids, labeled directly with HRP or indirectly with HRP-labeled antibodies/ streptavidin.

Advantages

- High sensitivity non-radioactive detection system.
- Stable hard copy results on film.
- Only small amounts of antibody required.
- Detection may be achieved in short exposure times (minutes).
- High resolution.

Figure 2: Principles of Protein Detection Procedure

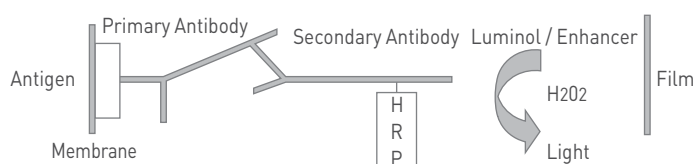
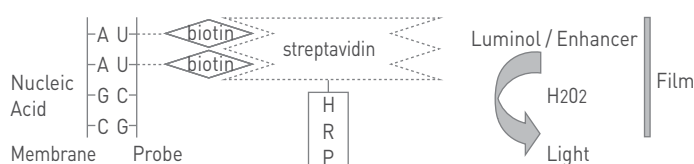


Figure 3: Principles of Nucleic Acid Detection Procedure



Kit Reagents

- Solution A, 60ml, contains: luminol and enhancer.
- Solution B, 60ml, contains: stable peroxide solution.

EZ-Hybridization Solution

Product Name	Catalogue No.	Unit Size
EZ-Hybridization Solution	01-889-1B	100ml

EZ-Hybridization Solution enables shorter hybridization times and decreases backgrounds. Therefore, low-copy RNA species on Northern blots and single-copy genes on Southern blots can be detected within 1-2 hours of hybridization using radioactively or non-radioactively labeled probes and for both cDNA and oligonucleotide probes.

Storage

- Room temperature.
- For long term storage, store at 2-8°C.

EZ-Block

Product Name	Catalogue No.	Unit Size
EZ-Block, (Blocking reagent for hybridization reactions and Western Blots)	41-805-10	10gr

EZ-Block is used in hybridization and detection procedures using nonradioactive nucleic acid probes, and for Western Blots.

When immunoassays and hybridization assays, such as dot blots, Western blots, Southern blots, or Northern blots are performed, there is nonspecific binding resulting in high background. In order to reduce the nonspecific binding, EZ-Block reagent is used to "block" unbound sites left after immobilization of the specific protein or after the hybridization with nonradioactive probe. EZ-Block improves sensitivity and reduces background.

Storage

- Room Temperature.

EZ-First Strand cDNA Synthesis Kit for RT-PCR

Product Name	Catalogue No.	Unit Size
EZ-First Strand cDNA Synthesis Kit, for RT-PCR	20-800-50	50 reactions

Premixed solutions for the synthesis of single-stranded cDNA from RNA for use as a PCR template.

The Polymerase Chain Reaction (PCR) is a powerful technique for rapid amplification of genes. In addition to amplifying genomic DNA template, PCR can also be used to amplify cDNA reverse transcribed from RNA to analyze gene expression. Using the EZ-First Strand cDNA Synthesis Kit, RNA is reverse transcribed into single-stranded cDNA. The reverse transcriptase (RT) enzyme synthesizes the new cDNA strand at a site determined by the type of primer used: Oligo(dT) primer, random primer or a sequence-specific primer. The first strand cDNA can then be used as a template for PCR.

The reaction mix solution supplied contains buffer, site-directed mutant of MMLV reverse transcriptase and RNase inhibitor sufficient for 50 reactions.

The other ready to use solution contains DTT, oligo dT primer, random primer, control RNA, control primers mix for PCR and DEPC-treated water.

Kit Reagents

1	RT Reaction Mix Contains: Reverse transcriptase, RNase inhibitor and dNTP's in buffer solution	400µl
2	DTT Solution, 100mM	100µl
3	Oligo (dT) 20 Primer, 40µM	50µl
4	Random Hexamer Primer, 40µM	50µl
5	Control RNA Human, Total RNA, 1µg/µl	25µl
6	Primer Mix Human G3PDH amplimers, 10µM each	50 µl
7	DEPC-Treated Water	1.5ml

Storage

- The control RNA should be stored at -70°C.
- The other premixed solutions should be stored at -20°C.

Quick-Load

Product Name	Catalogue No.	Unit Size	Storage Temp.
Quick Load 5X Conc. (PCR Loading Solution)	01-892-1H	5ml	2-8°C

A PCR loading solution for the direct loading of reaction products onto agarose gel.

Advantages

Use of Quick Load, which is a 5x loading dye solution containing a PCR compatible dye and an inert densifying agent, has all of the following advantages:

- Samples can be visualized while loading.
- PCR products can be loaded onto agarose gel immediately following amplification.
- Red dye serves as a marker dye during gel electrophoresis.
- Saves time.
- Easy to use.
- Ideal for 96-well PCR.
- Non-toxic ingredients.

DEPC-Treated Water

Product Name	Catalogue No.	Unit Size	Storage Temp.
DEPC-Treated Water	01-852-1A	500ml	AMB

Random Primer DNA Labeling Mix

Product Name	Catalogue No.	Unit Size
Random Primer DNA Labeling Mix	20-101-25	25 assays

Premixed solution for the labeling of DNA with radiolabeled dCTP using random sequence oligonucleotides.

The use of a "random primed" DNA sequence to prime DNA synthesis was originally introduced by Feinberg and Vogelstein. The method is based on the hybridization of oligonucleotides of all possible sequences to the denatured template DNA to be labeled. The complementary DNA strand is synthesized by a "Klenow" fragment of DNA Polymerase I, using the random oligonucleotides as primers. By substituting a radiolabeled nucleotide for a non-radioactive equivalent in the reaction mixture, the newly synthesized complementary DNA is made radioactive. The labeling mix system is a specially developed reaction mixture for enhanced convenience and performance. The reaction mixture contains random oligonucleotides, a Klenow fragment of DNA Polymerase I, dATP, dGTP, dTTP and a reaction buffer concentrate. The DNA labeling mix allows the labeling of the template DNA to a specific activity of 2x10⁹ dpm/µg after only 10 minutes of incubation. This rapid labeling is accomplished with the use of the Klenow fragment, which lacks 5'-3' exonuclease activity, and by the use of nonamer primers giving more efficient priming from the template at 37°C. The labeling mix method enables the labeling of small amounts of DNA (10-20ng), such as restriction fragments isolated from gels. Fragments can be labeled directly in low melting temperature agarose gel slices. The labeled probes are used in various hybridization techniques, such as Southern and Northern blots, in-situ hybridization and screening of gene libraries.

Kit reagents

1 vial containing 100µl DNA labeling mixture. Each vial is sufficient for 25 labeling assays.

Storage

The premixed solution should be stored at -20°C. Avoid repeated changes in the solution temperature.

DNA Isolation Kit

Product Name	Catalogue No.	Unit Size
DNA Isolation Kit	20-200-300	150-300 isolations

The DNA isolation kit provides a convenient method for extracting DNA from agarose gels, isolating plasmid DNA from mini-preps, as well as for concentration of DNA without ethanol precipitation.

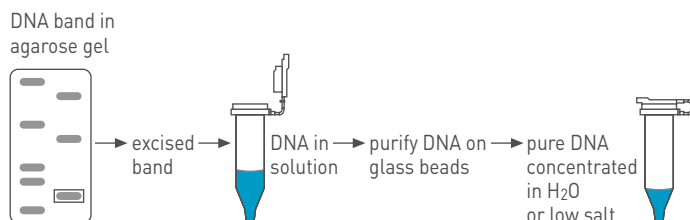
Kit reagents

- Glass powder suspension, 1.5ml.
- Sodium Iodide solution (6M), 120ml.
- Concentrated wash solution, 25ml.

Storage

- Sodium Iodide solution: store at 2-8°C, protect from light.
- Concentrated wash solution: store at -20°C in a glass bottle.

Figure 4: Purifying DNA from TAE agarose gel



Water Saturated Phenol

Product Name	Catalogue No.	Unit Size
Water Saturated Phenol pH recovery buffer for pH 7.9 included For use in RNA/DNA extraction	01-860-1L	250ml

Biological Industries Phenol is a liquid phase water-saturated phenol. It has an acid pH value, which allows direct use for RNA extraction. The water-saturated phenol does not contain any additive or antioxidant and it is packed under argon.

For DNA extraction, the pH of the water-saturated phenol has to be adjusted to pH 7.9 with the pH recovery buffer solution supplied.

Procedure: pH adjustment to 7.9

1. Add the 50ml pH Recovery Buffer Solution directly to the 250ml water saturated phenol.
2. Mix thoroughly and let the phases separate.
3. Use the neutralized phenol under the aqueous phase.

Storage

Water-saturated phenol should be stored at 2-8°C. If necessary, the phenol may be kept frozen at -20°C for long period. Protect the phenol from light.

Ultra Pure Water (DNase and RNase-free)

Product Name	Catalogue No.	Unit Size	Storage Temp.
Ultra Pure Water	01-866-1A	500ml	AMB
(DNase and RNase-free)	01-866-1B	100ml	AMB

TAE Buffer 50X Conc.

Product Name	Catalogue No.	Unit Size	Storage Temp.
TAE Buffer 50X Conc.	01-870-1A	500ml	AMB

TBE Buffer 50X Conc.

Product Name	Catalogue No.	Unit Size	Storage Temp.
TBE Buffer 5X Conc.	01-871-1A	500ml	AMB

Acrylamide / Bis-Acrylamide (T=40%)

Product Name	Catalogue No.	Unit Size	Storage Temp.
Acrylamide/Bis-Acrylamide (19:1 ratio) (T=40%)	01-872-1A	500ml	2-8°C
Acrylamide/Bis-Acrylamide (29:1 ratio) (T=40%)	01-874-1A	500ml	2-8°C
Acrylamide/Bis-Acrylamide (37.5:1 ratio) (T=40%)	01-876-1A	500ml	2-8°C

Polyacrylamide Gels

Polyacrylamide is a commonly used electrophoresis matrix for size separation of proteins and nucleic acids. The gel matrix is formed by free radical polymerization of acrylamide and a comonomer crosslinker (bis-acrylamide).

The gel pore size is determined by two parameters:

- Total monomer concentration (%T)
- The weight percentage of crosslinker (%C)

$$\%T = \frac{\text{gram (acrylamide + bis-acrylamide)}}{\text{Total volume (ml)}} \times 100$$

$$\%C = \frac{\text{gram (bis-acrylamide)}}{\text{gram (acrylamide + bis-acrylamide)}} \times 100$$

Gel with T=20% is prepared with 20% of acrylamide and bis-acrylamide. As %T is higher, the pore size of the gels are smaller.

Gel with T=20%, C=5% is prepared with 20% of acrylamide and bis-acrylamide and the weight percentage of the bis-acrylamide is 5% from the total weight of the acrylamide and bisacrylamide.

Biological Industries' acrylamide and bis-acrylamide solutions are available in 3 different crosslinker ratios: 19:1, 29:1 and 37.5 :1.

Standard protocol for SDS-PAGE gel

The solutions (10ml resolving gel and 10ml stacking gel may be used for the preparation of two gels with the size of 100mmx80mmx1.4mm.

Final concentration of gel	Resolving gel 10ml			Stacking gel 10ml
	7%	10%	12.5%	5%
Acrylamide-bis solution (T=40%)	1.75ml	2.5ml	3.13ml	1.25ml
Resolving buffer, 4x (w/o SDS)	2.5ml	2.5ml	2.5ml	---
Stacking buffer, 4x (w/o SDS)	---	---	---	2.5ml
Distilled water	5.65ml	4.9ml	4.27ml	6.15ml
SDS, 10% solution	0.1ml	0.1ml	0.1ml	0.1ml
TEMED	0.015ml	0.015ml	0.015ml	0.015ml
Ammonium persulphate, 10% solution	0.03ml	0.03ml	0.03ml	0.03ml

SDS Solution (10%)

Product Name	Catalogue No.	Unit Size	Storage Temp.
SDS Solution (10%)	01-890-1B	100ml	AMB

Kits for Molecular Biology

Product Name	Catalogue No.	Unit Size	Storage Temp.
Random Primer DNA Labeling Mix	20-101-25	25 assays	-20°
DNA Isolation Kit	20-200-300	150-300 isolations	2-8°C
EZ-RNA Total RNA Isolation Kit	20-400-100	100ml	2-8°C
EZ-RNA II Total RNA Isolation Kit Without Chloroform, with BCP	20-410-100	100ml	2-8°C
EZ-ECL Kit	20-500-120	1200cm ² (120ml)	2-8°C
EZ-DNA Genomic DNA Isolation Kit	20-600-50	50ml	AMB
EZ-First Strand cDNA Isolation Kit, for RT-PCR	20-800-50	50 reactions	-20°C

Auxiliary products

Product Name	Catalogue No.	Unit Size	Storage Temp.
DEPC-Treated Water	01-852-1A	500ml	AMB
Water Saturated Phenol, PH recovery buffer for PH 7.9 included For use in RNA/DNA extraction	01-860-1L	250ml	2-8°C
Ultra Pure Water (DNase and RNase-free)	01-866-1A 01-866-1B	500ml 100ml	AMB AMB
TAE Buffer 50X Conc.	01-870-1A	500ml	AMB
TBE Buffer 5X Conc.	01-871-1A	500ml	AMB
Acrylamide/Bis-Acrylamide (19:1 ratio) (T=40%)	01-872-1A	500ml	2-8°C
Acrylamide/Bis-Acrylamide (29:1 ratio) (T=40%)	01-874-1A	500ml	2-8°C
Acrylamide/Bis-Acrylamide (37.5:1 ratio) (T=40%)	01-876-1A	500ml	2-8°C
RBC Lysis Solution	01-888-1B	100ml	2-8°C
EZ-Hybridization Solution	01-889-1B	100ml	AMB
SDS Solution (10%)	01-890-1B	100ml	AMB
RNA Save	01-891-1A 01-891-1B 01-891-1C	500ml 100ml 20ml	2-8°C 2-8°C 2-8°C
Quick Load 5X Conc. (PCR Loading Solution)	01-892-1H	5ml	2-8°C
EZ Plant	01-893-1D	10ml	2-8°C
EZ Blood	01-894-1B	100ml	2-8°C
EZ-Block	41-805-10	10gr	AMB
Rnase-ExitusPlus™	01-897-1B 01-897-1L	100ml 250ml	AMB AMB

Human Serum and Blood Products

15 Human Serum and Blood Products

Biological Industries' Pre-Screened and Pre-tested human serum and blood products undergo the most stringent and rigorous Quality Control/Assurance Standards and Protocols testing all raw materials and finished products in order to meet the demands of international markets and ensure high quality and consistency.

All our human serum and blood products meet approved compliance validation and specifications prior to use and/or release of the final product to the end-user.

All our human serum and blood products undergo a methodical and comprehensive battery of physico-chemical, microbiological and biological performance testing procedures. Each batch is traceable, well-documented from source of origin through the thorough and systematic Quality Control process. All documentation and certification are available upon request.

Selecting the appropriate Human Serum Proteins depends upon at least four key factors:

- The cell line.
- The type of culture system.
- The chemical composition of the basal medium.
- The experience of the researcher.

Human Serum Albumin (HSA)

Product Name	Catalogue No.	Unit Size	Storage Temp.
Bio-Pure Human Serum Albumin (HSA Solution, 10%), Optimized for Human Embryonic, Stem Cells (hESC)	05-720-1B	100ml	-20°C
	05-720-1E	50ml	-20°C

HSA is a medium supplement that is a highly soluble osmolytic protein with a high molecular weight. It was specifically developed to support and maintain cell development, growth, health and productivity in most cell culture media, and especially cell membrane stability. The primary function of HSA is not only its unique demonstrative capability of binding anionic, cationic and neutral molecules, but it also has the proclivity of sequestering and stabilizing a wide array of ions and other small molecules.

HSA complies with the specifications of the manufacturer and the requirements stipulated by FDA approved tests.

All individual donations of the plasma and the corresponding plasma pool, has been tested for Hepatitis B Surface Antigen (HBsAg), Anti (Human Immunodeficiency Virus) HIV-I and II and anti-HCV and found to be negative.

Transferrin, Human, Substantially Iron-Free (APO) & Transferrin, Human, Iron-Saturated (HOLO)

Product Name	Catalogue No.	Unit Size	Storage Temp.
Transferrin, Human, Substantially Iron-Free (APO)	41-951-100	100mg	2-8°C
	41-951-500	500mg	2-8°C
Transferrin, Human, Iron-Saturated (HOLO)	41-952-100	100mg	2-8°C
	41-952-500	500mg	2-8°C

Transferrin is an important constituent in growth medium. It is a glycoprotein, but also known more specifically, as an iron-storage protein, found in mammalian serum (i.e. a blood plasma protein). Transferrin receptors, on the cell surface of actively growing cells, bind transferrin for iron transport to and from cells. In humans, it is still the most dynamically important iron pool relative to the total iron throughout the body. Nevertheless, research has shown that the majority of circulating iron-bound transferrin is transported to the bone marrow and incorporated into newly formed red blood cells or erythrocytes. The other primary storage depots for stored iron are the liver and spleen. The sum of all iron-binding sites on transferrin constitutes the total iron-binding capacity or TIBC of plasma. When iron-free, transferrin is known as apo-Transferrin and when iron saturated it is called holo-Transferrin. Traditionally, transferrin has entered the cell culture domain as a component of serum and is not a routine component of most commercially produced basal media. However, it has gained popularity and it is often added to classical basal media for the delivery of iron especially in a serum-free milieu. As a plasma-derived product, it is available for cell culture and diagnostic assays but not for therapeutic use.

Each unit of Biological Industries' transferrin is pasteurized and heated for 10 Hours @60°C. It is manufactured under GMP conditions from Human Blood Plasma sub Fraction IV-1. It is important to emphasize that it is for research, laboratory or further manufacturing purposes only. It is not intended for human use. It is a USA-sourced and approved product and plasma donors undergo a rigorous selection process as per FDA requirements.

Each unit of plasma and each plasma pool has been tested to be non-reactive for Hepatitis B Surface Antigen (HBsAg), Antibody to (Human Immunodeficiency Virus) HIV-I and II, anti-HCV and Syphilis.

EZ Lympho-Sep™-Lymphocyte Separation Tubes

Ready-to-use

Alternative to the "home made" blood separation tube

Density gradient centrifugation of whole blood on a polysucrose - sodium metrizoate medium is the method of choice for isolation of lymphocytes. The success of the procedure, i.e. the recovery of viable lymphocytes with the lowest proportion of contaminating granulocytes and erythrocytes, depends to a large extent on the careful layering of the blood sample onto the separation medium and the maintenance of a sharp interface between the two solutions prior to centrifugation. The EZ Lympho-Sep™ system allows the blood sample to be poured directly into the centrifuge tube with no special precautions required to prevent disruption of the polysucrose - sodium metrizoate layer. Thus, a large number of samples may be handled at the same time. The mechanism also reduces the length of centrifugation time required for separation of the lymphocytes.

The heart of the EZ Lympho-Sep™ is a plastic insert that allows the blood sample to be poured directly into the tube alleviating the need for slow and careful addition of the blood. Secondly, a one-way feature of the insert allows passage of materials during the centrifugation step but prevents the flow of the separation medium during shipping. After centrifugation, the upper lymphocyte-containing fraction may be poured off without risk of contamination from the erythrocytes, which are trapped under the insert.

EZ Lympho-Sep™ are ready-to-use sterile tubes with the separation medium already in place, and the one-way valve opening during centrifugation.

We offer four different sizes:

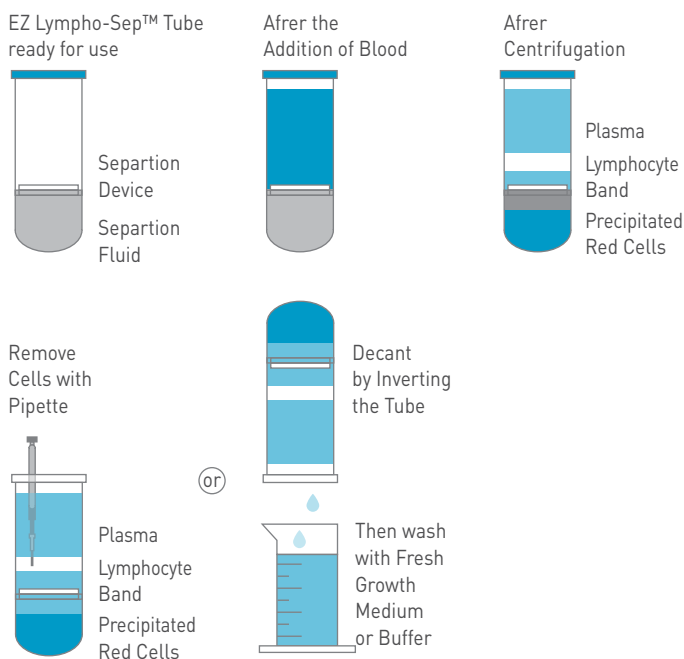
Catalogue No.	Separation Medium	Centrifuge Tube	Packaging
01-899-U02	2ml	15ml	30 Tubes/Box
01-899-U04	3ml	15ml	30 Tubes/Box
01-899-U10	10ml	50ml	18 Tubes/Box
01-899-U16	15ml	50ml	18 Tubes/Box

We can also supply empty tubes for customers who have their own media.

Features

- Ready-to-use, sterile.
- Safe method, minimum contact with biological fluids.
- Time saver, quick and easy sample filling.
- Maximum yield of viable mononuclear cells.
- A large number of samples may be handled at the same time.

Figure 1: The EZ Lympho-Sep™ system



Human Blood Products

- Tested negative or non reactive by FDA licensed tests for infectious disease markers: anti-HIV 1 & 2, HBsAG, anti-HCV, and other selected markers.
- Each product is traceable back to the donor, and additional information such as age, gender and geographic information is available upon request. (fresh units available for overnight shipments).
- Each donor is verbally and physically screened to comply with the most stringent FDA regulations. Products are obtained from volunteer and paid donors throughout the US.
- Pricing is based upon quantity ordered and information requested.
- Supply agreements and long-term contracts are available.

Human plasma

- EDTA Plasma
- Heparinized Plasma
- Fresh Frozen Plasma

Human whole Blood Products

- Off the clot
- Off the clot Male
- Off the clot Female

Processed Serum Production

The facility is solely for human production and does not process or package any animal products.

All human products are manufactured in a GMP facility FDA Regulation # 307234900.

Some of the processing capabilities include, but are not limited to:

- Defibrination
- Lipid stripping (several methods available)
- Charcoal stripping
- Diafiltration
- Large scale filtration
- Heat inactivation

Every stage of production, from receipt of raw material to the final QA review of finished product are carried out by highly trained staff according to established procedures.

Each type of plasma (or natural clot serum) used in the production of serum has its own Raw Material Specification (RMS) document on file to ensure that only the highest quality plasma is used in production. In addition to the initial inspection upon receiving, all shipments of plasma receive a 100% inspection for visual clarity and color against controlled standards. This attention to detail at the raw material stage and with control of our collections ensures lot-to-lot consistency for production of the highest quality finished product available.

For custom manufacturing contact us with your specifications or acceptance criteria for pricing and production lead time.

- Pooled Human Plasma.
- Human Serum – Defibrinated Plasma.
- Delipidized Human Serum.
- Defibrinated / Delipidized Serum.
- Defibrinated / Delipidized / Charcoal Stripped.
- Charcoal Stripped Human Serum.
- Serology Negative Serum.
- Defibrinated Plasma – Hepatitis Negative.
- Individual Human Serum from whole blood.
- Pooled Human Serum.

Serum Definitions

Defibrinated Serum: Serum derived from plasma by processing with chemicals.

Defibrinated, Delipidized Serum: Serum derived from plasma, processed to remove lipids. Optically clear.

Defibrinated, Delipidized, Charcoal Stripped Serum: Serum derived from plasma, processed to remove lipids and hormone proteins.

Defibrinated, Charcoal Stripped Serum: Serum derived from plasma, processed to remove hormone proteins but leaving lipids present.

Serology Negative Serum: Serum screened and found negative for serology disease state markers, screened and found negative for serology disease state markers.

Hepatitis Negative Serum: Serum derived from plasma where it is necessary for the bulk pool to be negative (non-reactive) for numerous viral markers by Elisa and PCR methodology. Suitable for negative control matrices.

Human Serum (OTC): Whole blood is collected from pre qualified human donors. Red cells are separated by centrifuge and serum is naturally clotted. Serum is stored in plastic bottles at $\leq -20^{\circ}\text{C}$

Related products

Product Name	Catalogue No.	Unit Size	Storage Temp.
EZ-DNA Genomic DNA Isolation Kit	20-600-50	50ml	AMB
EZ Blood	01-894-1B	100 ml	2-8°C
RBC Lysis Solution	01-888-1B	100ml	2-8°C
Phytohemagglutinin-M (PHA-M), Liquid	12-009-1H	5ml	-20°C
Human Fibronectin Solution, 1mg/ml	05-750-1H	1ml	2-8°C
	05-750-1F	5ml	2-8°C

Appendixes

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Worldwide Distributors
Certifications
Representation of companies in the Domestic Market
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Numerical Index
Terms & Conditions

16 Appendixes Formulations

Basal Medium-Eagle (BME)⁽¹⁾

Product Name Catalogue No. Component	BME Earle's Salt Base 01-015-1* mg/liter	BME 10x Earle's Salt Base 01-015-5*+ mg/liter
CaCl ₂ ·2H ₂ O	264.92	2649.2
KCl	400.0	4000.0
KH ₂ PO ₄	-	-
MgCl ₂ ·6H ₂ O	-	-
MgSO ₄ ·7H ₂ O	200.0	2000.0
NaCl	6800.0	68000.0
NaHCO ₃	2200.0	-
NaH ₂ PO ₄ ·H ₂ O	140.0	1400.0
Na ₂ HPO ₄ ·7H ₂ O	-	-
D-GLUCOSE	1000.0	10000.0
PHENOL RED	10.0	100.0
L-ARGININE HCl	21.0	210.0
L-CYSTINE	12.0	120.0
L-GLUTAMINE	292.0*	2920.0*
L-HISTIDINE HCl·H ₂ O	10.5	105.0
L-ISOLEUCINE	26.0	260.0
L-LEUCINE	26.0	260.0
L-LYSINE HCl	36.5	365.3
L-METHIONINE	7.5	75.0
L-PHENYLALANINE	16.5	165.0
L-THREONINE	24.0	240.0
L-TRYPTOPHAN	4.0	40.0
L-TYROSINE	18.0	180.0
L-VALINE	23.5	235.0
D-BIOTIN	1.0	10.0
D-CALCIUM PANTOTHENATE	1.0	10.0
CHOLINE CHLORIDE	1.0	10.0
FOLIC ACID	1.0	10.0
i-INOSITOL	2.0	20.0
NICOTINAMIDE	1.0	10.0
PYRIDOXAL HCl	1.0	10.0
RIBOFLAVIN	0.1	1.0
THIAMINE HCl	1.0	10.0

* This preparation is without L-Glutamine.

+ All media concentrates 10X are prepared without Sodium Bicarbonate.

⁽¹⁾ Eagle, H., Exptl. Soc. Biol. Med., 89:362, (1955).

Modified Eagle's Minimum Essential Medium

Dulbecco's Modified Eagle's Media(1) (DMEM) Iscove's Modified Dulbecco's Media(2) (IMDM)

Product Name Catalogue No. Component	DMEM 1X Low Gluc. 01-050-1* mg/liter	DMEM 5X Low Gluc. 01-050-4*+ mg/liter	DMEM 1X High Gluc. 01-055-1* mg/liter	DMEM 5X High Gluc. 01-055-4*+ mg/liter	ISCOVE'S 1X 01-058-1 mg/liter
CaCl ₂ ·2H ₂ O	264.92	1324.6	264.92	1324.6	218.6
Fe(NO ₃) ₃ ·9H ₂ O	0.1	0.5	0.1	0.5	-
KCl	400.0	2000.0	400.0	2000.0	330.0
KNO ₃	-	-	-	-	0.076
MgSO ₄ ·7H ₂ O	200.0	1000.0	200.0	1000.0	200.0
NaCl	6400.0	32000.0	6400.0	32000.0	4505.0
NaHCO ₃	3700.0	-	3700.0	-	3024.0
NaH ₂ PO ₄ ·H ₂ O	125.0	625.0	125.0	625.0	125.0
NaSeO ₃ ·5H ₂ O	-	-	-	-	0.0173
D-GLUCOSE	1000.0	5000.0	4500.0	22500.0	4500.0
PHENOL RED	15.0	75.0	15.0	75.0	15.0
SODIUM PYRUVATE	110.0	550.0	-	-	110.0
HEPES	-	-	-	-	5958.0
L-ALANINE	-	-	-	-	25.0
L-ARGININE HCl	84.0	420.0	84.0	420.0	84.0
L-ASPARAGINE·H ₂ O	-	-	-	-	28.4
L-ASPARTIC ACID	-	-	-	-	30.0
L-CYSTINE	48.0	240.0	48.0	240.0	70.0
L-GLUTAMIC ACID	-	-	-	-	75.0
L-GLUTAMINE	584.0*	2920.0*	584.0*	2920.0*	584.0*
GLYCINE	30.0	150.0	30.0	150.0	30.0
L-HISTIDINE HCl·H ₂ O	42.0	210.0	42.0	210.0	42.0
L-ISOLEUCINE	105.0	525.0	105.0	525.0	105.0
L-LEUCINE	105.0	525.0	105.0	525.0	105.0
L-LYSINE HCl	146.0	730.0	146.0	730.0	146.0
L-METHIONINE	30.0	150.0	30.0	150.0	30.0
L-PHENYLALANINE	66.0	330.0	66.0	330.0	66.0
L-PROLINE	-	-	-	-	40.0
L-SERINE	42.0	210.0	42.0	210.0	42.0
L-THREONINE	95.0	475.0	95.0	475.0	95.0
L-TRYPTOPHAN	16.0	80.0	16.0	80.0	16.0
L-TYROSINE	72.0	360.0	72.0	360.0	72.0
L-VALINE	94.0	470.0	94.0	470.0	94.0

Product Name	DMEM 1X Low Gluc.	DMEM 5X Low Gluc.	DMEM 1X High Gluc.	DMEM 5X High Gluc.	ISCOVE'S 1X
Catalogue No. Component	01-050-1* mg/liter	01-050-4*+ mg/liter	01-055-1* mg/liter	01-055-4*+ mg/liter	01-058-1 mg/liter
D-BIOTIN	-	-	-	-	0.013
D-CALCIUM PANTOTHENATE	4.0	20.0	4.0	20.0	4.0
CHOLINE CHLORIDE	4.0	20.0	4.0	20.0	4.0
FOLIC ACID	4.0	20.0	4.0	20.0	4.0
i-INOSITOL	7.2	36.0	7.2	36.0	7.2
NICOTINAMIDE	4.0	20.0	4.0	20.0	4.0
PYRIDOXINE HCl	4.0	20.0	4.0	20.0	4.0
RIBOFLAVIN	0.4	2.0	0.4	2.0	0.4
THIAMINE HCl	4.0	20.0	4.0	20.0	4.0
VITAMIN B ₁₂	-	-	-	-	0.013

* This preparation is without L-Glutamine.

+ All media concentrates 5X are prepared without Sodium Bicarbonate.

⁽¹⁾ Dulbecco, R. and Freeman, G., Virology, 8:396, (1959).

Smith, J.D. et al, Virology, 12:185, (1960).

TCA Standards Committee, In Vitro, Vol. 6/2:93, (1970).

⁽²⁾ Iscove, N.N. and Melchers, F., Jour. of Exp. Med., Vol. 147:923.

Ribonucleosides and Deoxyribonucleosides for MEM Alpha (500x Concentrate)

Catalogue No. 01-343-1	mg/ml
ADENOSINE	5.0
CYTIDINE	5.0
GUANOSINE	5.0
URIDINE	5.0
2'-DEOXYADENOSINE	5.0
2'-DEOXYCYTIDINE HCl	5.5
2'-DEOXYGUANOSINE	5.0
2'-DEOXYTHYMIDINE	5.0

Minimum Essential Medium Alpha

Catalogue No. 01-042-1 Component	mg/liter	Catalogue No. 01-042-1 Component	mg/liter
CaCl ₂ ·2H ₂ O	265.0	L-LEUCINE	52.4
Fe(NO ₃) ₃ ·9H ₂ O	-	L-LYSINE HCl	72.4
KCl	400.0	L-METHIONINE	15.0
MgSO ₄ ·7H ₂ O	200.0	L-PHENYLALANINE	32.0
NaCl	6800.0	L-PROLINE	40.0
NaHCO ₃	2200.0	L-SERINE	25.0
NaH ₂ PO ₄ ·H ₂ O	140.0	L-THREONINE	48.0
D-GLUCOSE	1000.0	L-TRYPTOPHAN	10.0
PHENOL RED	10.0	L-TYROSINE	36.0
SODIUM PYRUVATE	110.0	L-VALINE	46.0
LIPOIC ACID	0.2	L-ASCORBIC ACID	50.0
L-ALANINE	25.0	D-BIOTIN	0.1
L-ARGININE HCl	127.0	D-CALCIUM PANTOTHENATE	1.0
L-ASPARAGINE·H ₂ O	50.0	CHOLINE CHLORIDE	1.0
L-ASPARTIC ACID	30.0	FOLIC ACID	1.0
L-CYSTINE	24.0	i-INOSITOL	2.0
L-CYSTEINE HCl·H ₂ O	100.0	NICOTINAMIDE	1.0
L-GLUTAMIC ACID	75.0	PYRIDOXAL HCl	1.0
L-GLUTAMINE	292.0	RIBOFLAVIN	0.1
GLYCINE	50.0	THIAMINE HCl	1.0
L-HISTIDINE HCl·H ₂ O	42.0	TRYPTOPHOSPHATE BROTH	-
L-ISOLEUCINE	52.5	VITAMIN B ₁₂	1.36
L-LEUCINE	52.4		

* This preparation is without L-Glutamine.

+ All media concentrates 10X are prepared without Sodium Bicarbonate.

⁽¹⁾ Nature, New Biology 230:310, (1971).

⁽²⁾ MacPherson, J. and Stoker, M., Virology, 16:147, (1962).

Roswell Park Memorial Institute Tissue Culture Media Series

Product Name	RPMI 1640 ⁽¹⁾	RPMI 1640 ⁽¹⁾ 10x	RPMI 1640 With HEPES	McCOY'S 5A ⁽²⁾ Modified
Catalogue No. Component	01-100-1 mg/liter	01-104-5*+ mg/liter	01-106-1 mg/liter	01-075-1 mg/liter
CaCl ₂	-	-	-	100.0
Ca(NO ₃) ₂ ·4H ₂ O	100.0	1000.0	100.0	-
KCl	400.0	4000.0	400.0	400.0
MgSO ₄ ·7H ₂ O	100.0	1000.0	100.0	200.0
NaCl	6000.0	60000.0	5500.0	6460.0
NaHCO ₃	2000.0	-	2000.0	2200.0
Na ₂ HPO ₄ ·7H ₂ O	1512.0	15120.0	1512.0	-
NaH ₂ PO ₄ ·H ₂ O	-	-	-	580.0
D-GLUCOSE	2000.0	20000.0	2000.0	3000.0
PHENOL RED	5.0	50.0	5.0	5.0
L-ALANINE	-	-	-	13.4
L-ARGININE	200.0	2000.0	200.0	34.9
L-ASPARAGINE·H ₂ O	50.0	500.0	50.0	45.0
L-ASPARTIC ACID	20.0	200.0	20.0	19.9
L-CYSTEINE	-	-	-	31.5
L-CYSTINE	50.0	500.0	50.0	-
L-GLUTAMIC ACID	20.0	200.0	20.0	22.1
L-GLUTAMINE	300.0	3000.0*	300.0	219.2
GLUTATHIONE (Reduced)	1.0	10.0	1.0	0.5
GLYCINE	10.0	100.0	10.0	7.5
L-HISTIDINE	15.0	150.0	15.0	15.25
L-HYDROXYPROLINE	20.0	200.0	20.0	19.7
L-ISOLEUCINE	50.0	500.0	50.0	39.3
L-LEUCINE	50.0	500.0	50.0	39.3
L-LYSINE HCl	40.0	400.0	40.0	36.5
L-METHIONINE	15.0	150.0	15.0	14.9
L-PHENYLALANINE	15.0	150.0	15.0	16.5
L-PROLINE	20.0	200.0	20.0	17.3
L-SERINE	30.0	300.0	30.0	26.3
L-THREONINE	20.0	200.0	20.0	17.9
L-TRYPTOPHAN	5.0	50.0	5.0	3.1
L-TYROSINE	20.0	200.0	20.0	18.1
L-VALINE	20.0	200.0	20.0	17.6
ASCORBIC ACID	-	-	-	0.5
D-BIOTIN	0.2	2.0	0.2	0.2

Product Name	RPMI 1640 ⁽¹⁾	RPMI 1640 ⁽¹⁾ 10x	RPMI 1640 With HEPES	McCOY'S 5A ⁽²⁾ Modified
Catalogue No. Component	01-100-1 mg/liter	01-104-5*+ mg/liter	01-106-1 mg/liter	01-075-1 mg/liter
VITAMIN B ₁₂	0.005	0.05	0.005	2.0
D-CALCIUM PANTOTHENATE	0.25	2.5	0.25	0.2
CHOLINE CHLORIDE	3.0	30.0	3.0	5.0
FOLIC ACID	1.0	10.0	1.0	10.0
i-INOSITOL	35.0	350.0	35.0	36.0
NICOTINAMIDE	1.0	10.0	1.0	0.5
NICOTINIC ACID	-	-	-	0.5
p-AMINOBENZOIC ACID	1.0	10.0	1.0	1.0
PYRIDOXAL HCl	-	-	-	0.5
PYRIDOXINE HCl	1.0	10.0	1.0	0.5
RIBOFLAVIN	0.2	2.0	0.2	0.2
THIAMINE HCl	1.0	10.0	1.0	0.2
BACTO PEPTONE	-	-	-	600.0
HEPES	-	-	5958.0	-

Catalogue No. 01-100-1 RPMI-1640⁽¹⁾ with L-Glutamine

Catalogue No. 01-104-5*+ RPMI-1640⁽¹⁾ 10X without L-Glutamine

Catalogue No. 01-107-1 RPMI-1640 DUTCH Modification with L-Glutamine

Catalogue No. 01-075-1 McCOY'S 5A⁽²⁾ (Modified) with L-Glutamine

* This preparation is without L-Glutamine.

+ All media concentrates 10X are prepared without Sodium Bicarbonate.

⁽¹⁾ Moore, G., Gerner, R.E. and Franklin H.A., J.A.M.A., 199, (1967).

⁽²⁾ Iwakata, S. and Grace Jr. J.T., N.Y. State J. Med., 64/18, (1964)
(Similar to RPMI-1629 Medium).

Medium M-199

Product Name Catalogue No. Component	M-199 E Earle's Salts Base 01-080-1 mg/liter	M-199 E Earle's Salts Base 10x 01-080-5+ mg/liter	M-199 H Hank's Salts Base 01-085-1 mg/liter
CaCl ₂	200.0	2000.0	140.0
Fe(NO ₃) ₃ ·9H ₂ O	0.72	7.2	0.72
KCl	400.0	4000.0	400.0
KH ₂ PO ₄	-	-	60.0
MgSO ₄ ·7H ₂ O	200.0	2000.0	200.0
NaCl	6800.0	68000.0	8000.0
NaHCO ₃	2200.0	-	350.0
NaH ₂ PO ₄ ·H ₂ O	140.0	1400.0	-
Na ₂ HPO ₄ ·7H ₂ O	-	-	90.0
D-GLUCOSE	1000.0	10000.0	1000.0
PHENOL RED	20.0	200.0	20.0
ADENINE SULPHATE	10.0	100.0	10.0
ADENOSINE TRIPHOSPHATE Na ₄	1.0	10.0	1.0
ADENYLIC ACID	0.2	2.0	0.2
CHOLESTEROL	0.2	2.0	0.2
D-2-DEOXYRIBOSE	0.5	5.0	0.5
GLUTATHIONE (Reduced)	0.05	0.5	0.05
GUANINE HCl	0.3	3.0	0.3
HYPOXANTHINE	0.3	3.0	0.3
D-RIBOSE	0.5	5.0	0.5
SODIUM ACETATE	50.0	500.0	50.0
THYMINE	0.3	3.0	0.3
TWEEN 80	20.0	200.0	20.0
URACIL	0.3	3.0	0.3
XANTHINE	0.3	3.0	0.3
L-ALANINE	25.0	250.0	25.0
L-ARGININE HCl	70.0	700.0	70.0
L-ASPARTIC ACID	30.0	300.0	30.0
L-CYSTEINE HCl·H ₂ O	0.11	1.1	0.11
L-CYSTINE	20.0	200.0	20.0
L-GLUTAMIC ACID·H ₂ O	75.0	750.0	75.0
L-GLUTAMINE	100.0	1000.0	100.0
GLYCINE	50.0	500.0	50.0
L-HISTIDINE HCl·H ₂ O	21.88	218.8	21.88
L-HYDROXYPROLINE+	10.0	100.0	10.0
L-ISOLEUCINE	20.0	200.0	20.0

Product Name Catalogue No. Component	M-199 E Earle's Salts Base 01-080-1 mg/liter	M-199 E Earle's Salts Base 10x 01-080-5+ mg/liter	M-199 H Hank's Salts Base 01-085-1 mg/liter
L-LEUCINE	60.0	600.0	60.0
L-LYSINE HCl	70.0	700.0	70.0
L-METHIONINE	15.0	150.0	15.0
L-PHENYLALANINE	25.0	250.0	25.0
L-PROLINE	40.0	400.0	40.0
L-SERINE	25.0	250.0	25.0
L-THREONINE	30.0	300.0	30.0
L-TRYPTOPHAN	10.0	100.0	10.0
L-TYROSINE	40.0	400.0	40.0
L-VALINE	25.0	250.0	25.0
ASCORBIC ACID	0.05	0.5	0.05
ALPHA TOCOPHEROL PHOSPHATE Na ₂	0.01	0.1	0.01
D-BIOTIN	0.01	0.1	0.01
CALCIFEROL	0.1	1.0	0.1
D-CALCIUM PANTOTHENATE	0.01	0.1	0.01
CHOLINE CHLORIDE	0.5	5.0	0.5
FOLIC ACID	0.01	0.1	0.01
i-INOSITOL	0.05	0.5	0.05
MENADIONE	0.01	0.1	0.01
NIACIN	0.025	0.25	0.025
NIACINAMIDE	0.025	0.25	0.025
p-AMINOBENZOIC ACID	0.05	0.5	0.05
PYRIDOXAL HCl	0.025	0.25	0.025
PYRIDOXINE HCl	0.025	0.25	0.025
RIBOFLAVIN	0.01	0.1	0.01
THIAMINE HCl	0.01	0.1	0.01
VITAMIN A	0.1	1.0	0.1

+ All media concentrates 10X are prepared without Sodium Bicarbonate. Morgan, Morton and Parker, Proc. Soc. Biol. Med., 73:1, (1950).

Leibovitz L-15 Medium

Catalogue No. 01-115-1 Component	mg/liter	Catalogue No. 01-115-1 Component	mg/liter
CaCl ₂	140.0	L-LEUCINE	125.0
KCl	400.0	L-LYSINE	75.0
KH ₂ PO ₄	60.0	DL-METHIONINE	150.0
MgCl ₂ ·6H ₂ O	200.0	DL-PHENYLALANINE	250.0
MgSO ₄ ·7H ₂ O	200.0	L-SERINE	200.0
NaCl	8000.0	DL-THREONINE	600.0
Na ₂ HPO ₄	190.0	L-TRYPTOPHAN	20.0
D(+)-GALACTOSE	900.0	L-TYROSINE	300.0
PHENOL RED	10.0	DL-VALINE	200.0
SODIUM PYRUVATE	550.0	DL-CALCIUM PANTOTHENATE	1.0
DL-ALPHA ALANINE	450.0	CHOLINE CHLORIDE	1.0
L-ARGININE	500.0	FOLIC ACID	1.0
L-ASPARAGINE	250.0	i-INOSITOL	2.0
L-CYSTEINE	120.0	NICOTINAMIDE	1.0
L-GLUTAMINE	300.0	PYRIDOXINE HCl	1.0
GLYCINE	200.0	RIBOFLAVIN 5'PHOSPHATE, Na	0.1
L-HISTIDINE	250.0	THIAMINE HCl	1.0
DL-ISOLEUCINE	250.0		

Leibovitz, A., Am. J. Hyg., 78 (1963)

Minimum Essential Media - Eagle (Mem)

Product Name	MEM Earle's Salts Base	MEM Earle's Salts Base With Non-Essential Amino Acids	MEM Hank's Salts Base	MEM Earle's Salts Base Conc. 10x	MEM For Suspensions
Catalogue No. Component	01-025-1* mg/liter	01-040-1* mg/liter	01-035-1* mg/liter	01-025-5*+ mg/liter	01-045-1* mg/liter
CaCl ₂ ·2H ₂ O	264.92	264.92	185.44	2649.2	-
KCl	400.0	400.0	400.0	4000.0	400.0
KH ₂ PO ₄	-	-	60.0	-	-
MgCl ₂ ·6H ₂ O	-	-	100.0	-	-
MgSO ₄ ·7H ₂ O	200.0	200.0	100.0	2000.0	200.0
NaCl	6800.0	6800.0	8000.0	68000.0	6800.0
NaHCO ₃	2200.0	2200.0	350.0	-	2200.0
NaH ₂ PO ₄ ·H ₂ O	140.0	140.0	-	1400.0	1400.0
Na ₂ HPO ₄ ·7H ₂ O	-	-	90.0	-	-

Product Name	MEM Earle's Salts Base	MEM Earle's Salts Base With Non-Essential Amino Acids	MEM Hank's Salts Base	MEM Earle's Salts Base Conc. 10x	MEM For Suspensions
Catalogue No. Component	01-025-1* mg/liter	01-040-1* mg/liter	01-035-1* mg/liter	01-025-5*+ mg/liter	01-045-1* mg/liter
GLUCOSE	1000.0	1000.0	1000.0	10000.0	1000.0
PHENOL RED	10.0	10.0	10.0	100.0	10.0
L-ALANINE	-	8.9	-	-	-
L-ARGININE HCl	126.0	126.0	126.0	1260.0	126.0
L-ASPARAGINE-H ₂ O	-	15.0	-	-	-
L-ASPARTIC ACID	-	13.3	-	-	-
L-CYSTINE	24.0	24.0	24.0	240.0	24.0
L-GLUTAMIC ACID	-	14.7	-	-	-
L-GLUTAMINE	292.0*	292.0*	292.0*	-	292.0*
GLYCINE	-	7.5	-	-	-
L-HISTIDINE HCl·H ₂ O	42.0	42.0	42.0	420.0	42.0
L-ISOLEUCINE	52.0	52.0	52.0	520.0	52.0
L-LEUCINE	52.0	52.0	52.0	520.0	52.0
L-LYSINE HCl	72.5	72.5	72.5	725.0	72.5
L-METHIONINE	15.0	15.0	15.0	150.0	15.0
L-PHENYLALANINE	32.0	32.0	32.0	320.0	32.0
L-PROLINE	-	11.5	-	-	-
L-SERINE	-	10.5	-	-	-
L-THREONINE	48.0	48.0	48.0	480.0	48.0
L-TRYPTOPHAN	10.0	10.0	10.0	100.0	10.0
L-TYROSINE	36.0	36.0	36.0	360.0	36.0
L-VALINE	46.0	46.0	46.0	460.0	46.0
D-CALCIUM PANTOTHENATE	1.0	1.0	1.0	10.0	1.0
CHOLINE CHLORIDE	1.0	1.0	1.0	10.0	1.0
FOLIC ACID	1.0	1.0	1.0	10.0	1.0
i-INOSITOL	2.0	2.0	2.0	20.0	2.0
NICOTINAMIDE	1.0	1.0	1.0	10.0	1.0
PYRIDOXAL HCl	1.0	1.0	1.0	10.0	1.0
RIBOFLAVIN	0.1	0.1	0.1	1.0	0.1
THIAMINE HCl	1.0	1.0	1.0	10.0	1.0

* This preparation is without L-Glutamine.

+ All media concentrates 10X are prepared without Sodium Bicarbonate.

⁽¹⁾ Eagle, H. Science, 130:432(1959).

Nutrient Mixtures F-10, F-12, and DMEM: F-12

Nutrient Mixture Catalogue No. Component	F-10 ⁽¹⁾ 01-090-1 mg/liter	F-12 ⁽²⁾ 01-095-1 mg/liter	DMEM:F-12 (1:1) 01-170-1* mg/liter
CaCl ₂ ·2H ₂ O	44.1	44.0	154.76
CuSO ₄ ·5H ₂ O	0.0025	0.0025	0.00125
FeSO ₄ ·7H ₂ O	0.834	0.834	0.417
Fe(NO ₃) ₃ ·9H ₂ O	-	-	0.05
KCl	285.0	223.6	311.8
KH ₂ PO ₄	83.0	-	-
MgCl ₂ ·6H ₂ O	-	122.0	61.0
MgSO ₄ ·7H ₂ O	152.8	-	100.0
NaCl	7400.0	7599.0	6999.5
NaHCO ₃	1200.0	1176.0	1200.0
NaH ₂ PO ₄ ·H ₂ O	-	-	62.5
Na ₂ HPO ₄	153.7	142.04	71.02
ZnSO ₄ ·7H ₂ O	0.0288	0.863	0.4315
D-GLUCOSE	1100.0	1802.0	3151.0
HEPES	-	-	3575.0
HYPOXANTHINE	4.0	4.1	2.05
LINOLEIC ACID	-	0.084	0.042
LIPOIC ACID	0.2	0.2	0.1
PHENOL RED	1.2	1.2	8.1
PUTRESCINE 2HCl	-	0.161	0.0805
SODIUM PYRUVATE	110.0	110.0	55.0
THYMIDINE	0.7	0.73	0.365
L-ALANINE	9.0	8.9	4.45
L-ARGININE HCl	211.0	211.0	147.5
L-ASPARAGINE·H ₂ O	15.0	15.0	7.505
L-ASPARTIC ACID	13.3	13.3	6.65
L-CYSTEINE HCl·H ₂ O	35.12	35.12	17.56
L-CYSTINE	-	-	24.0
L-GLUTAMIC ACID	14.7	14.7	7.35
L-GLUTAMINE	146.0	146.0	365.0
GLYCINE	7.51	7.5	18.75
L-HISTIDINE HCl·H ₂ O	23.0	20.96	31.48
L-ISOLEUCINE	2.6	3.94	54.47
L-LEUCINE	13.0	13.1	59.05
L-LYSINE HCl	29.0	36.5	91.25
L-METHIONINE	4.48	4.48	17.24

Nutrient Mixture Catalogue No. Component	F-10 ⁽¹⁾ 01-090-1 mg/liter	F-12 ⁽²⁾ 01-095-1 mg/liter	DMEM:F-12 (1:1) 01-170-1* mg/liter
L-PHENYLALANINE	5.0	4.96	35.48
L-PROLINE	11.5	34.5	17.25
L-SERINE	10.5	10.5	26.25
L-THREONINE	3.57	11.9	53.45
L-TRYPTOPHAN	0.6	2.04	9.02
L-TYROSINE	1.8	5.4	38.7
L-VALINE	3.5	11.7	52.85
D-BIOTIN	0.024	0.0073	0.00365
D-CA PANTOTHENATE	0.715	0.48	2.24
CHOLINE CHLORIDE	0.698	13.96	8.98
FOLIC ACID	1.32	1.3	2.65
i-INOSITOL	0.541	18.0	12.6
NIACINAMIDE	0.615	0.037	2.02
PYRIDOXAL HCl	-	-	2.0
PYRIDOXINE HCl	0.206	0.062	0.031
RIBOFLAVIN	0.376	0.038	0.219
THIAMINE HCl	1.00	0.34	2.17
VITAMIN B ₁₂	1.36	1.36	0.68

* This preparation is without L-Glutamine.

⁽¹⁾ Ham, R.G., Exp. Cell Res., 29: 515-526, (1963).

⁽²⁾ Ham, R.G., Proc. Nat. Ac. Sci., 53: 288-293, (1965).

Waymouth's MB 752/1 Medium

Catalogue No. 01-110-1		Catalogue No. 01-110-1	
Component	mg/liter	Component	mg/liter
CaCl ₂ ·2H ₂ O	120.00	L-LEUCINE	50.00
KCl	150.00	L-LYSINE HCl	240.00
KH ₂ PO ₄	80.00	L-METHIONINE	50.00
MgCl ₂ ·6H ₂ O	240.00	L-PHENYLALANINE	50.00
MgSO ₄ ·7H ₂ O	200.00	L-PROLINE	50.00
NaCl	6000.00	L-THREONINE	75.00
NaHCO ₃	2240.00	L-TRYPTOPHAN	40.00
Na ₂ HPO ₄	300.00	L-TYROSINE	40.00
D-GLUCOSE	5000.00	L-VALINE	65.00
GLUTATHIONE	15.00	ASCORBIC ACID	17.50
HYPOXANTHINE	25.00	D-BIOTIN	0.02
PHENOL RED	10.00	D-CALCIUM PANTOTHENATE	1.00
L-ARGININE HCl	75.00	CHOLINE CHLORIDE	250.00
L-ASPARTIC ACID	60.00	FOLIC ACID	0.40
L-CYSTEINE HCl·H ₂ O	100.26	i-INOSITOL	0.90
L-CYSTINE	15.00	NICOTINAMIDE	1.00
L-GLUTAMIC ACID	150.00	PYRIDOXINE HCl	1.00
L-GLUTAMINE	350.00	RIBOFLAVINE	1.00
GLYCINE	50.00	THIAMINE HCl	10.00
L-HISTIDINE HCl·H ₂ O	164.10	VITAMIN B ₁₂	0.20
L-ISOLEUCINE	25.00		

Waymouth, Ch., J. Nat. Canc. Inst., 22:1003, (1959).

Schneider's Drosophila Medium

Catalogue No. 01-150-1 with L-Glutamine		Catalogue No. 01-150-1 with L-Glutamine	
Component	mg/liter	Component	mg/liter
CaCl ₂ (anhyd.)	600.00	L-CYSTEINE	60.00
KCl	1600.00	L-CYSTINE	100.00
KH ₂ PO ₄	450.00	L-GLUTAMIC ACID	800.00
MgSO ₄ ·7H ₂ O	3700.00	L-GLUTAMINE	1800.00
NaCl	2100.00	GLYCINE	250.00
NaHCO ₃	400.00	L-HISTIDINE	400.00
Na ₂ HPO ₄ ·7H ₂ O	1321.00	L-ISOLEUCINE	150.00
Alpha-KETOGLUTARIC ACID	200.00	L-LEUCINE	150.00
FUMARIC ACID	100.00	L-LYSINE HCl	1650.00
D-GLUCOSE	2000.00	L-METHIONINE	800.00

Catalogue No. 01-150-1 with L-Glutamine		Catalogue No. 01-150-1 with L-Glutamine	
Component	mg/liter	Component	mg/liter
MALIC ACID	100.00	L-PHENYLALANINE	150.00
SUCCINIC ACID	100.00	L-PROLINE	1700.00
TREHALOSE	2000.00	L-SERINE	250.00
YEASTOLATE	2000.00	L-THREONINE	350.00
β-ALANINE	500.00	L-TRYPTOPHAN	100.00
L-ARGININE	400.00	L-TYROSINE	500.00
L-ASPARTIC ACID	400.00	L-VALINE	300.00

Schneider, I., J. Exp. Zool., 156 (1964).

Grace's Insect Cell Medium

Catalogue No. 01-155-1 with L-Glutamine		Catalogue No. 01-155-1 with L-Glutamine	
Component	mg/liter	Component	mg/liter
CaCl ₂ ·2H ₂ O	1324.00	L-ISOLEUCINE	50.00
KCl	2240.00	L-LEUCINE	75.00
MgCl ₂ ·6H ₂ O	2280.00	L-LYSINE HCl	625.00
MgSO ₄ ·7H ₂ O	2780.00	L-LYSINE HCl	625.00
NaHCO ₃	350.00	L-METHIONINE	50.00
NaH ₂ P ₀ 4·H ₂ O	1013.00	L-PHENYLALANINE	150.00
Alpha-KETOGLUTARIC ACID	370.00	L-PROLINE	350.00
FRUCTOSE	400.00	DL-SERINE	1100.00
FUMARIC ACID	55.00	L-THREONINE	175.00
D-GLUCOSE	700.00	L-TRYPTOPHAN	100.00
MALIC ACID	670.00	L-TYROSINE	50.00
SUCCINIC ACID	60.00	L-VALINE	100.00
SUCROSE	26680.00	D-BIOTIN	0.01
β-ALANINE	200.00	D-CALCIUM PANTOTHENATE	0.02
L-ALANINE	225.00	CHOLINE CHLORIDE	0.20
L-ARGININE HCl	700.00	FOLIC ACID	0.02
L-ASPARAGINE	350.00	i-INOSITOL	0.02
L-ASPARTIC ACID	350.00	NIACIN	0.02
L-CYSTINE	22.00	p-AMINOBENZOIC ACID	0.02
L-GLUTAMIC ACID	600.00	PYRIDOXINE HCl	0.02
L-GLUTAMINE	600.00	RIBOFLAVIN	0.02
GLYCINE	650.00	THIAMINE HCl	0.02
L-HISTIDINE	2500.00		

Grace, T.C.C., NATURE, 195:788 (1962).

Balanced Salt Solutions

Product Concentration Catalogue No. Component	1. Earle's Balanced Salt Solution		2. Hanks' Balanced Salt Solution		3. Spinner Modified Salt Solution
	1x 02-010-1 g/liter	10x 02-010-5+ g/liter	1x 02-015-1 g/liter	10x 02-015-5+ g/liter	1x 02-030-1 g/liter
CaCl ₂	0.2	2.0	0.14	1.4	-
KCl	0.4	4.0	0.4	4.0	0.4
KH ₂ PO ₄	-	-	0.06	0.6	-
MgCl ₂ ·6H ₂ O	-	-	0.1	1.0	-
MgSO ₄ ·7H ₂ O	0.2	2.0	0.1	1.0	0.2
NaCl	6.8	68.0	8.0	80.0	6.8
NaHCO ₃	2.2	-	0.35	-	2.2
Na ₂ HPO ₄ ·7H ₂ O	-	-	0.09	0.9	-
NaH ₂ PO ₄ ·H ₂ O	0.14	1.4	-	-	1.4
D-GLUCOSE	1.0	10.0	1.0	10.0	1.0
PHENOL RED	0.01	0.1	0.01	0.1	0.01

Product Concentration Catalogue No. Component	4. Dulbecco's Phosphate Buffered Saline			5. Alsever's Solution
	1x 02-023-1 g/liter	10x 02-023-5 g/liter	1x 02-020-1 g/liter	1x 02-045-1 g/liter
CaCl ₂	-	-	0.1	-
KCl	0.2	2	0.2	-
KH ₂ PO ₄	0.2	2	0.2	-
MgCl ₂ ·6H ₂ O	-	-	0.1	-
NaCl	8.0	80.0	8.0	4.2
Na ₂ HPO ₄ ·7H ₂ O	2.17	21.7	2.17	-
SODIUM CITRATE·2H ₂ O	-	-	-	8.0
D-GLUCOSE	-	-	-	20.5
PHENOL RED	-	-	-	-

+ This product is prepared without Sodium Bicarbonate.

1. Earle, W.R. et al., Natl. Cancer Inst., 4:167. (1943).
2. Hanks, J.H. and Wallace R.E., Proc. Soc. Exp. Biol. Med., 71:196, (1949).
3. Eagle, H., Science 130:432, (1959).
4. Dulbecco, R. and Voght M., J. Exp. Med., 98:167, (1954)
5. Chanock, R.M. and Sabin, A.B., J. Immunol., 70:271 (1953).

Concentrated Component Solutions

Amino Acids and Vitamins

Product Concentration Catalogue No. Component	BME Amino Acids 100x 01-315-1 g/liter	BME Vitamins 100x 01-316-1 g/liter	MEM Amino Acids 50x 01-325-1 g/liter	MEM Vitamins 100x 01-326-1 g/liter	MEM Non- Essential Amino Acids 100x 01-340-1 g/liter
	NaCl	-	8.50	-	8.50
L-ALANINE	-	-	-	-	0.89
L-ARGININE HCl	2.10	-	6.32	-	-
L-ASPARAGINE·H ₂ O	-	-	-	-	1.50
L-ASPARTIC ACID	-	-	-	-	1.33
D-BIOTIN	-	0.10	-	-	-
D-CALCIUM PANTOTHENATE	-	0.10	-	0.10	-
CHOLINE CHLORIDE	-	0.10	-	0.10	-
L-CYSTINE	1.20	-	1.20	-	-
FOLIC ACID	-	0.10	-	0.10	-
L-GLUTAMIC ACID	-	-	-	-	1.47
GLYCINE	-	-	-	-	0.75
L-HISTIDINE HCl·H ₂ O	1.05	-	2.10	-	-
i-INOSITOL	-	0.20	-	0.20	-
L-ISOLEUCINE	2.60	-	2.62	-	-
L-LEUCINE	2.60	-	2.62	-	-
L-LYSINE HCl	3.65	-	3.62	-	-
L-METHIONINE	0.75	-	0.75	-	-
NICOTINAMIDE	-	0.10	-	0.10	-
L-PHENYLALANINE	1.65	-	1.65	-	-
L-PROLINE	-	-	-	-	1.15
PYRIDOXAL HCl	-	0.10	-	0.10	-
RIBOFLAVIN	-	0.01	-	0.01	-
L-SERINE	-	-	-	-	1.05
THIAMINE HCl	-	0.10	-	0.10	-
L-THREONINE	2.40	-	2.38	-	-
L-TRYPTOPHAN	0.40	-	0.51	-	-
L-TYROSINE	1.80	-	1.80	-	-
L-VALINE	2.35	-	2.34	-	-

Powdered Cell Culture Media Formulations

Product	MEM- EARLE'S 11-025-1 mg/liter	MEM- ALPHA 11-042-1 mg/liter	DMEM LOW GLUCOSE 11-050-1 mg/liter	DMEM HIGH GLUCOSE 11-055-1 mg/liter
Calcium Chloride Dihydrate	264.90	264.90	264.90	264.90
Ferric Nitrate Nonahydrate	-	-	0.10	0.10
Potassium Chloride	400.00	400.00	400.00	400.00
Magnesium Sulfate	97.66	97.66	97.66	97.66
Sodium Chloride	6800.00	6800.00	6400.00	6400.00
Sodium Dihydrogen Phosphate	121.74	121.74	108.69	108.69
L-Alanine	-	25.00	-	-
L-Arginine Hydrochloride	126.98	126.98	84.00	84.00
L-Asparagine Monohydrate	-	50.00	-	-
L-Aspartic Acid	-	30.00	-	-
L-Cysteine Hydrochloride Monohydrate	-	100.00	-	-
L-Cystine Dihydrochloride	31.29	31.29	62.58	62.58
L-Glutamic Acid	-	75.00	-	-
L-Glutamine	292.00	292.00	584.00	584.00
Glycine	-	50.00	30.00	30.00
L-Histidine Hydrochloride Monohydrate	42.00	42.00	42.00	42.00
L-Isoleucine	52.00	52.50	104.80	104.80
L-Leucine	52.00	52.50	104.80	104.80
L-Lysine Hydrochloride	72.46	72.46	146.20	146.20
L-Methionine	15.00	15.00	30.00	30.00
L-Phenylalanine	32.00	32.00	66.00	66.00
L-Proline	-	40.00	-	-
L-Serine	-	25.00	42.00	42.00
L-Threonine	48.00	48.00	95.20	95.20
L-Tryptophan	10.00	10.00	16.00	16.00
L-Tyrosine Disodium	51.90	51.90	103.79	103.79
L-Valine	46.00	46.00	93.60	93.60
Ascorbic Acid	-	50.00	-	-
D-Biotin	-	0.10	-	-
Choline Chloride	1.00	1.00	4.00	4.00
Folic Acid	1.00	1.00	4.00	4.00
i-Inositol	2.00	2.00	7.00	7.00
Nicotinamide	1.00	1.00	4.00	4.00
D-Calcium Pantothenate	1.00	1.00	4.00	4.00
Pyridoxal Hydrochloride	1.00	1.00	4.00	4.00

Product	MEM- EARLE'S 11-025-1 mg/liter	MEM- ALPHA 11-042-1 mg/liter	DMEM LOW GLUCOSE 11-050-1 mg/liter	DMEM HIGH GLUCOSE 11-055-1 mg/liter
Riboflavin	0.10	0.10	0.40	0.40
Thiamine Hydrochloride	1.00	1.00	4.00	4.00
Vitamin B-12	-	1.36	-	-
D-Glucose	1000.00	1000.00	1000.00	4500.00
Lipoic Acid	-	0.20	-	-
Phenol Red Sodium	10.20	10.20	15.34	15.34
Sodium Pyruvate	-	110.00	110.0	-

Powdered Cell Culture Media Formulations

Product	M-199 EARLE'S 11-080-1 mg/liter	NUT. F-10 11-090-1 mg/liter	NUT. F-12 11-095-1 mg/liter	DMEM F-12 (1:1) 11-170-1 mg/liter
Calcium Chloride Dihydrate	264.90	44.10	44.10	154.52
Cupric Sulfate Pentahydrate	-	0.0025	0.002	0.00125
Ferric Nitrate Nonahydrate	0.72	-	-	0.05
Ferrous Sulfate Heptahydrate	-	0.834	0.834	0.417
Potassium Chloride	400.00	285.00	223.65	311.83
Potassium Dihydrogen Phosphate	-	83.00	-	-
Magnesium Chloride	-	-	57.20	28.60
Magnesium Sulfate	97.66	74.62	-	48.83
Sodium Bicarbonate	-	-	-	-
Sodium Chloride	6800.00	7400.00	7599.00	6999.50
Sodium Dihydrogen Phosphate	121.74	-	-	54.35
Disodium Hydrogen Phosphate	-	153.73	142.12	71.06
Zinc Sulfate Heptahydrate	-	0.0288	0.863	0.432
L-Alanine	25.00	8.91	8.91	4.45
L-Arginine Hydrochloride	70.00	211.00	210.7	147.35
L-Asparagine	-	-	15.01	-
L-Asparagine Monohydrate	-	15.00	-	7.51
L-Aspartic Acid	30.00	13.30	13.30	6.66
L-Cysteine Hydrochloride Monohydrate	0.11	39.10	35.12	17.56
L-Cystine Dihydrochloride	26.08	-	-	31.29
L-Glutamic Acid	75.00	14.70	14.71	7.36

Product Catalogue No. Component	M-199 EARLE'S 11-080-1 mg/liter	NUT. F-10 11-090-1 mg/liter	NUT. F-12 11-095-1 mg/liter	DMEM F-12 (1:1) 11-170-1 mg/liter
L-Glutamine	100.00	146.20	146.20	365.10
Glycine	50.00	7.51	7.51	18.76
L-Histidine Hydrochloride Monohydrate	21.88	22.98	20.96	31.48
Hydroxy-L-Proline	10.00	-	-	-
L-Isoleucine	20.00	2.60	3.936	54.37
L-Leucine	60.00	13.10	13.10	58.96
L-Lysine Hydrochloride	70.00	29.30	36.54	91.37
L-Methionine	15.00	4.48	4.48	17.24
L-Phenylalanine	25.00	4.96	4.96	35.48
L-Proline	40.00	11.50	34.53	17.27
L-Serine	25.00	10.50	10.50	26.26
L-Threonine	30.00	3.57	11.91	53.56
L-Tryptophan	10.00	0.60	2.042	9.02
L-Tyrosine Disodium	57.66	2.61	7.836	55.81
L-Valine	25.00	3.50	11.71	52.66
Ascorbic Acid	0.05	-	-	-
Biotin	0.01	0.024	0.007	0.00367
Choline Chloride	0.50	0.697	13.96	8.98
Ergocalciferol	0.10	-	-	-
Folic Acid	0.01	1.32	1.32	2.662
i-Inositol	0.05	0.541	18.02	12.51
Menadione	0.01	-	-	-
Nicotinamide	0.025	0.615	0.037	2.018
Nicotinic Acid	0.025	-	-	-
D-Calcium Pantothenate	0.01	0.715	0.238	2.12
p-Aminobenzoic Acid	0.05	-	-	-
Pyridoxal Hydrochloride	0.025	-	-	2.00
Pyridoxine Hydrochloride	0.025	0.206	0.062	0.031
Riboflavin	0.01	0.376	0.038	0.22
Thiamine Hydrochloride	0.01	1.012	0.337	2.17
Vitamin A Acetate	0.14	-	-	-
Vitamin B-12	-	1.36	1.36	0.679
Vitamin E Phosphate Disodium	0.01	-	-	-
Adenine Sulfate	10.00	-	-	-
Adenosine Triphosphate Sodium	1.00	-	-	-
Adenylic Acid	0.20	-	-	-

Product Catalogue No. Component	M-199 EARLE'S 11-080-1 mg/liter	NUT. F-10 11-090-1 mg/liter	NUT. F-12 11-095-1 mg/liter	DMEM F-12 (1:1) 11-170-1 mg/liter
Cholesterol	0.20	-	-	-
Deoxyribose	0.50	-	-	-
D-Glucose	1000.00	1100.00	1801.60	3150.80
Glutathione (Reduced)	0.05	-	-	-
Guanine	0.30	-	-	-
Hypoxanthine Sodium	0.399	5.43	5.43	2.715
Linoleic Acid	-	-	0.084	0.0421
Lipoic Acid	-	0.20	0.206	0.103
Phenol Red (Sodium)	20.40	1.23	1.27	8.305
Putrescine Dihydrochloride	-	-	0.161	0.0806
D-Ribose	0.50	-	-	-
Sodium Acetate	50.00	-	-	-
Sodium Pyruvate	-	110.00	110.10	55.05
Thymidine	-	0.727	0.727	0.360
Thymine	0.30	-	-	-
Tween 80	20.00	-	-	-
Uracil	0.30	-	-	-
Xanthine Sodium	0.344	-	-	-
Hepes	-	-	-	15mM

Powdered Cell Culture Media Formulations

Product	McCOY'S 5A	ISCOVE'S	MEM With NEAA	RPMI 1640
Catalogue No. Component	11-075-1 mg/liter	11-058-1 mg/liter	11-040-1 mg/liter	11-100-1 mg/liter
Calcium Chloride Dihydrate	132.46	218.56	264.86	-
Calcium Nitrate Tetrahydrate	-	-	-	100.00
Potassium Chloride	400.00	330.00	400.00	400.00
Potassium Nitrate	-	0.076	-	-
Potassium Dihydrogen Phosphate	-	-	-	-
Magnesium Chloride	-	-	-	-
Magnesium Sulfate	97.66	97.66	97.66	48.83
Na ₂ SeO ₃ ·5H ₂ O	-	0.0173	-	-
Sodium Chloride	6460.00	4505.00	6800.00	6000.00
Sodium Dihydrogen Phosphate	504.35	108.69	121.73	-
Disodium Hydrogen Phosphate	-	-	-	800.49
DL-Alanine	-	-	-	-
L-Alanine	13.90	25.00	8.90	-
L-Arginine	-	-	-	-
L-Arginine Hydrochloride	42.10	84.00	126.40	241.86
L-Asparagine	45.00	-	-	-
L-Asparagine Monohydrate	-	28.40	15.00	50.00
L-Aspartic Acid	19.97	30.00	13.30	20.00
L-Cysteine	31.50	-	-	-
L-Cysteine Hydrochloride Monohydrate	-	-	-	-
L-Cystine Dihydrochloride	-	91.24	28.39	65.19
L-Glutamic Acid	22.10	75.00	14.70	20.00
L-Glutamine	219.20	584.00	292.00	300.00
Glycine	7.50	30.00	7.50	10.00
L-Histidine Hydrochloride Monohydrate	20.96	42.00	41.93	20.27
Hydroxy-L-Proline	19.70	-	-	20.00
L-Isoleucine	39.36	104.80	52.00	50.00
L-Leucine	39.36	104.80	52.00	50.00
L-Lysine	-	-	-	-
L-Lysine Hydrochloride	36.50	146.20	73.06	40.00
DL-Methionine	-	-	-	-
L-Methionine	14.90	30.00	15.00	15.00

Product	McCOY'S 5A	ISCOVE'S	MEM With NEAA	RPMI 1640
Catalogue No. Component	11-075-1 mg/liter	11-058-1 mg/liter	11-040-1 mg/liter	11-100-1 mg/liter
DL-Phenylalanine	-	-	-	-
L-Phenylalanine	16.50	66.00	33.00	15.00
L-Proline	17.30	40.00	11.50	20.00
L-Serine	26.30	42.00	10.50	30.00
DL-Threonine	-	-	-	-
L-Threonine	17.90	95.20	48.00	20.00
DL-Tryptophan	-	-	-	-
L-Tryptophan	3.10	16.00	10.00	5.00
L-Tyrosine	-	-	-	-
L-Tyrosine Disodium	26.10	103.79	44.74	28.83
DL-Valine	-	-	-	-
L-Valine	17.60	93.60	46.86	20.00
Ascorbic Acid	0.50	-	-	-
D-Biotin	0.20	0.013	-	0.20
Choline Chloride	5.00	4.00	1.00	3.00
Folic Acid	10.00	4.0	1.00	1.00
i-Inositol	36.00	7.00	2.00	35.00
Nicotinamide	0.50	4.00	1.00	1.00
Nicotinic Acid	0.50	-	-	-
D-Calcium Pantothenate	0.20	4.00	1.00	0.25
p-Aminobenzoic Acid	1.00	-	-	1.00
Pyridoxal Hydrochloride	0.50	4.00	1.00	-
Pyridoxine Hydrochloride	-	-	-	1.00
Riboflavin	0.20	0.40	0.10	0.20
Riboflavin 5'-Phosphate	-	-	-	-
Thiamine Hydrochloride	0.20	4.00	1.00	1.00
Thiamine Monophosphate Dihydrate	-	-	-	-
Vitamin B-12	2.00	0.013	-	0.005
Bactopectone	600.00	-	-	-
D(+) Galactose	-	-	-	-
D-Glucose	3000.00	4500.00	1000.00	2000.00
Glutathione (Reduced)	0.50	-	-	1.00
Hypoxanthine Sodium	-	-	-	-
Phenol Red (Sodium)	10.20	15.34	10.00	5.1
Sodium Pyruvate	-	110.00	-	-
HEPES	-	25 mM	-	-

Crystalline Trypsin Solution & Soybean Trypsin Inhibitor Solution

Product	CRYSTALLINE TRYPSIN Solution	SOYBEAN TRYPSIN INHIBITOR
Catalogue No. Component	03-047-1 gm/l	03-048-1 gm/l
CRYSTALLINE TRYPSIN	0.2	-
SOYBEAN TRYPSIN INHIBITOR	-	5.0
POLYVINYL PYRROLIDONE	5.0	-
TRICINE	3.58	-
NaCl	8.0	8.0
KCl	0.4	0.2
Na ₂ HPO ₄ 7H ₂ O	-	2.16
KH ₂ PO ₄	-	0.2
PHENOL RED	-	-

Trypsin Solutions

Product	TRYPSIN Solution "A"	TRYPSIN Solution "B"	TRYPSIN EDTA Solution "A"	TRYPSIN EDTA Solution "B"	TRYPSIN EDTA Solution "C"	TRYPSIN Solution "B" Conc. 10x	TRYPSIN EDTA Conc. 10x
Catalogue No. Component	03-045-1 gm/l	03-046-1 gm/l	03-050-1 gm/l	03-052-1 gm/l	03-053-1 gm/l	03-046-5 gm/l	03-051-5 gm/l
TRYPSIN 1:250	2.5	2.5	2.5	2.5	0.5	25.0	5.0
EDTA Na ₂ 2H ₂ O	-	-	0.2	0.5	0.2	-	2.0
NaCl	8.0	8.0	8.0	8.0	8.0	80.0	8.5
KCl	0.2	0.2	0.4	0.4	0.2	2.0	-
Na ₂ HPO ₄	1.15	1.15	-	-	1.15	11.5	-
KH ₂ PO ₄	0.2	0.2	-	-	0.2	2.0	-
D-GLUCOSE	-	-	1.0	1.0	-	-	-
PHENOL RED	-	-	0.01	0.01	0.01	-	-
NaHCO ₃	-	-	0.35	0.35	-	-	-
CaCl ₂ ·2H ₂ O	0.13	-	-	-	-	-	-
MgCl ₂ ·6H ₂ O	0.1	-	-	-	-	-	-

16 Appendixes Worldwide Distributors



Austria

Bio Products

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e office@bioproducts.at

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Pragostem s.r.o

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e mail@cosmobio.co.jp
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f 03-5632-9619



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Palestinian Authority

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Medical & General Trading Company Ltd.

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16 Appendixes Certifications

IQNet
THE INTERNATIONAL CERTIFICATION NETWORK

CERTIFICATE
IQNet and THE STANDARDS INSTITUTION OF ISRAEL hereby certify that the organization

Biological Industries Israel Beit Haemek Ltd. KIBBUTZ BEIT HAEMEK, ISRAEL
for the following field of activities

- DESIGN, MANUFACTURE AND DISTRIBUTION OF:
A. RESEARCH PRODUCTS FOR CELL CULTURE, CELL BIOLOGY AND MOLECULAR BIOLOGY, B. MEDICAL DEVICES INCLUDING IN-VITRO DIAGNOSTIC PRODUCTS, C. CELL CULTURE REAGENTS FOR INDUSTRIAL BIO-PROCESSING.
- CONTRACT MANUFACTURING OF RESEARCH PRODUCTS AND MEDICAL DEVICES INCLUDING IN-VITRO DIAGNOSTIC PRODUCTS.

has implemented and maintains a
Management System
which fulfills the requirements of the following standard's
ISO 13485:2003

Issued on: 27. 03. 2013
Date of expiration: 20. 05. 2013
Date of initial approval: 10. 04. 2008
Registration number: IL- 48546

Michael Drecher
President of IQNet

Daniel Goldstein
Director General, SII

www.sii.org.il

IQNet
THE INTERNATIONAL CERTIFICATION NETWORK

CERTIFICATE
IQNet and THE STANDARDS INSTITUTION OF ISRAEL hereby certify that the organization

Biological Industries Israel Beit Haemek Ltd. KIBBUTZ BEIT HAEMEK
for the following field of activities
MANUFACTURE OF PRODUCTS FOR ANIMAL CELL CULTURE AND MOLECULAR BIOLOGY
(LABORATORY TESTING SERVICES)
(SEE APPENDIX)

has implemented and maintains a
Quality Management System
which fulfills the requirements of the following standard's
ISO 9001:2008

Issued on: 08. 04. 2010
Date of expiration: 20. 05. 2013
Date of initial approval: 07. 02. 1995
Registration number: IL- 55837

Rena Haimov
President of IQNet

Doron Tamir
Director General, SII

www.sii.org.il

16 Appendixes Representation of Companies in the Domestic Market

Biological Industries represent several leading Life Science research, diagnostic and bioprocess providers to local clients.

Abnova (www.abnova.com)

Abnova is the world's largest antibody manufacturer. They have the capacity of generating 300 mouse monoclonal antibodies and 200 rabbit polyclonal antibodies per month. Rather than the traditional method of antibody production, Abnova is taking a genomic/proteomic approach for the antibody development.

Applichem AG (www.applichem.com/home)

AppliChem supplies chemicals for chemical, biological, pharmaceutical and clinical research.

ATCC (www.atcc.org)

ATCC is a private, nonprofit biological resource center (BRC) and research organization whose mission focuses on the acquisition, authentication, production, preservation, development and distribution of standard reference microorganisms, cell lines and other materials for research in the life sciences.

ChemoMetec (www.chemometec.com)

Cell counting and cell analysis.

Devyser AB (www.devyser.com)

Devyser specializes in diagnostic kits and reagents based on DNA analytical procedures, including PCR. The main areas of application for these products are prenatal diagnostics and clinical genetics.

Fitzco (www.fitzcoinc.com)

Forensic and DNA collection

Fitzco's forensic division carries a complete line of evidence packaging products including: evidence pouches, needle storage tubes, heat impulse sealers, and custom labeling.

Herolab (<http://herolab.de/>)

The core competence of Herolab is based in Centrifuges with the necessary accessories and its ability to meet the needs of any application – from the small table top centrifuge to the high speed floor model with a capacity of up to 6 liters.

IDEA Bio-Medical (www.idea-bio.com)

IDEA Bio-Medical has developed a high definition, ultra-fast biological cell-imaging system WiScan™ capable of high resolution imaging at high speeds.

Implen (<http://implen.com>)

Implen is a privately held corporation that is a leading supplier for spectroscopy instruments and consumables for the non-destructive analysis of ultra low volume samples. The company focuses on biological, chemical, and pharmaceutical laboratories in industry and research.

Kreatech (www.kreatech.com)

A molecular diagnostics company focused on innovative detection products. These are used for diagnostic and research applications in the life sciences and healthcare industry. These applications include cytogenetics, microarrays, and proteomics.

Has a particular expertise in the areas of oncology, haematology and pre-natal diagnostics.

Has a well established range of FISH probes and offers a new range of repeat-free probes for superior results in cytogenetic analysis.

ProFoldin (www.promega.com)

Protein preparation and assay technologies.

Promega (www.promega.com)

Molecular Biology Leadership

Leading products for Life science research, particularly in genomics, proteomics, and cellular analysis. Promega's products are also used to support molecular diagnostics and human identification.

Promega's products for advanced molecular biology research are a part of Biological Industries' integrative approach of providing a full service package to academia and biopharma customers in the local market. We have a long term mutually beneficial relationship with Promega with the constant provision of innovation and interactive technical support at the highest level.

Promega's loyal customers in Israel in clinical and academic labs serve as beta sites for new developments and product introductions. Several research collaborations were catalyzed by BI between Israeli scientists and Promega.

New: MaxWell- a user friendly microrobotic system from Promega has been well recognized as a solution for high throughput DNA testing.

Promocell AG (www.promocell.com)

PromoCell is a premier manufacturer of cell culture products and is especially recognized for its broad range of human primary cells, stem cells and blood cells, as well as optimized cell culture media. Scientists worldwide use PromoCell products in basic and applied biomedical research to obtain better results from more accurate physiological models.

Under its PromoKine brand, PromoCell also offers a wide range of well proven products for cell biology research such as cell analysis kits, cell transfection reagents, fluorescent dyes, antibodies, ELISAs, and cytokines which help scientists to facilitate their daily lab work and obtain optimal results.

Sartorius-Stedim (www.sartorius.com)**Global Leader in Bioprocess and Filtration Applications**

Sartorius is one of the world's leading providers of laboratory and process technologies and equipment. Its key customers are from the biotech, pharma and food industries, as well as from public research institutes and laboratories. Sartorius operates its own production facilities in Europe, Asia and America, and also has sales offices and local representatives in more than 110 countries. Strongly rooted in the scientific and research communities and closely allied with customers and technology partners, the company is dedicated to its philosophy of "Turning science into solutions" every single day.

Stemgent (www.stemgent.com)

Stemgent, Inc. is engaged in working alongside some of the world's leading stem cell scientists in developing innovative technology and application solutions for the advancement of stem cell research. Its mission is to help simplify and support cellular reprogramming research by producing products being designed by leading stem cell researchers worldwide. Its scientific development sites are located in both Cambridge, Massachusetts and San Diego, California.

Shun Tai (www.yip.com.hk/shuntai)

Specializes in manufacturing all types of working gloves.

16 Appendixes Alphabetical Index

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A		
Acrylamide/Bis-Acrylamide (19:1 ratio) (T=40%)	01-872-1	87
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Acrylamide/Bis-Acrylamide (37.5:1 ratio) (T=40%)	01-876-1	87
Adult Bovine Serum	04-003-1	53
Adult Bovine Serum, Heat Inactivated	04-123-1	53
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DMEM Powder, with 4.5g/l D-Glucose (High Glucose), without Sodium Pyruvate, with L-Glutamine, without Sodium Bicarbonate	11-055-1	37, 38, 103
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16 Appendixes Term & Conditions

1. ACCEPTANCE

1.a The terms and conditions of sale contained herein apply to all quotations made and purchase orders entered into by the Seller. Some of the terms set out here may differ from those in Buyer's purchase order and some may be new. This acceptance is conditional on Buyer's assent to the terms set out here in lieu of those in Buyer's purchase order. Seller's failure to object to provisions contained in any communication from Buyer shall not be deemed a waiver of the provisions of this acceptance. Any change in the terms contained herein must be specifically agreed to in writing by an officer of the Seller before becoming binding on either the Seller or the Buyer. These terms shall be applicable whether or not they are attached to or enclosed with the products to be sold or sold hereunder.

2. PAYMENT

- 2.a All invoices are due and payable thirty (30) days from the date of Air Waybill. No discounts are authorized. Shipments, Deliveries, and performance of work shall at all times be subject to the approval of the Seller and the Seller may at any time decline to make any shipments or deliveries or perform any work except upon receipt of payment or upon terms and conditions of security satisfactory to the Seller.
- 2.b If, in the Judgment of the Seller, the financial condition of buyer at any time does not justify continuation of production or shipment on the terms of payment originally specified, the Seller may require full or partial payment in advance and in the event of the bankruptcy or insolvency of the Buyer or in the event any proceeding is brought by or against the Buyer under; bankruptcy or insolvency laws, the Seller shall be entitled to cancel any order then outstanding and shall receive reimbursement for its cancellation charges.
- 2.c Each shipment shall be considered a separate and independent transaction, and payment therefore shall be made accordingly. If shipments are delayed by the Buyer, with consent of the Seller, payments shall become due on the date when the Seller is prepared to make shipment. If the work covered by the purchase order is delayed by the Buyer, with consent of the Seller, payments shall be made based on the purchase price and the percentage of completion. Products held for the buyer shall be at the risk and expense of the Buyer.
- 2.d Payment will be made in Israel according to the Seller's instructions. Without derogating from any right or remedy the Seller may have under law any amount not paid on time and in full shall bear interest from the date it became due and until the date of actual payment, at the maximum interest rate for the time prevailing at Bank Hapoalim in Israel.

3. TAXES

3.a The amount of any present or future sales, revenue, excise or other taxes, fees, or other charges of any nature, imposed by any public authority (national, state, local or other) applicable to the Products covered by the order, or the manufacturer or sale thereof, shall be added to the purchase price and shall be paid by the Buyer, or in lieu thereof, the Buyer shall provide the Seller with a tax exemption certificate acceptable to the taxing authority.

4. DELIVERY

- 4.a Where a period is named for delivery the Buyer shall take delivery within that period, unless otherwise expressly agreed by the parties in writing. The Buyer shall notify the Seller within 3 days of the arrival of the products at the port.
- 4.b Any time or date for delivery named by the Seller is an estimate only and is based upon prompt receipt from Buyer of all necessary information. In no event shall Seller be liable for re-procurement costs, nor for delay or non-delivery, due to causes beyond its reasonable control including, but not limited to, acts of civil or military authority, priorities, fires, strikes, lockouts, slowdowns, shortages, factory or labor conditions, errors in manufacture and inability due to causes beyond the Seller's reasonable control to obtain necessary labor, materials or manufacturing facilities. In the event of any such delay, the date of delivery shall at the request of the Seller, be deferred for a period equal to the time lost by reason of the delay.
- 4.c The Buyer at his own expense shall make provisions for the transport and/or collection of the Products from any port to which they may be sent by the Seller.

5. PASSING OF RISK AND PASSING OF TITLE

- 5.a From the date of arrival of the Products, as such date is specified in the Air Waybill, the risk of any loss or damage or deterioration of the Products due to whatever cause, shall be borne by the Buyer.
- 5.b The title in the Products shall remain vested in the Seller until the full and total consideration thereof shall have been paid to the Seller. Until the said full and total payment, the Buyer shall hold, at its expense, the Products on behalf of the Seller as bailee.

6. WARRANTY

6.a The Seller warrants that the products are free from defects in material and workmanship under normal use and service. Seller's obligations under this warranty are limited to replacing or giving credit for, at its option, at its factory, any of said Products which are after examination, disclosed to the Seller's satisfaction to be thus defective and which shall, at the Seller's option, be returned within 14 days after demand to the Seller's factory of origin, transportation charges repaid. The alleged defect, if any, shall not be a ground for cancellation of the remainder of the contract or order. This Warranty is expressed in lieu of all other warranties, expressed, statutory, or implied, including the implied warranties

of merchantability and fitness for a particular purpose, and of all other obligations or liabilities on the Seller's part, and it neither assumes nor authorizes any other person to assume for the Seller any other liabilities in connection with the said Products or the sale thereof. This Warranty shall not apply to any Products which shall be subjected to misuse, negligence, or accident. Liability of the Seller, if any hereunder, shall in no event exceed in amount of the purchase price of the Products sold with respect to which claims are made. Seller neither assumes nor authorizes any person to assume for it any other liability in connection with the sale or use of the Products sold hereunder, and there are no oral agreements or warranties collateral with or affecting this Agreement Seller shall not for any reason whatsoever be liable for loss of production or profits, or for other consequential losses which may be suffered or alleged to have been suffered by Buyer.

- 6.b The replacement or the giving of credit in accordance with the provisions of this paragraph above is subject to the procedure dictated by the Seller's insurance policy regarding the same and to the complete and accurate compliance by the Buyer with the instructions given by the Seller regarding the same.

7. CLAIMS

- 7.a No claims for damage in transit or loss of Products shall be entertained unless a complete claim in writing is given to the carrier concerned, and to the Seller and its agent (if any) within such time as will enable the compliance with the carrier's conditions of carriage as affecting damage in transit or loss of Products. Where Products are accepted from the carrier concerned without being checked, the delivery book of the carrier concerned must be signed "not examined".

8. DISTRIBUTORS

- 8.a For current information on Biological industries distributors and sales offices, please see distributors list in the appendix or use our website, www.bioind.com, or contact your local Biological Industries distributor to place your order. In the event that you do not have an authorized distributor, please contact:

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9. TECHNICAL SUPPORT

- 9.a Providing excellent technical support for our products is our top priority. Our technical service team relies on years of laboratory experience to assist you with product selection and advise to maximize product performance. You may reach our technical support via telephone/fax or email (see on the reverse side of the catalogue).

10. ONLINE ORDERING

- 10.a Online ordering for all of our products is available on our website www.bioind.com. Our online ordering system is secure, fast, and convenient.

11. GENERAL

- 11.a The sale of the Products by the Seller to the Buyer is subject to the length of the shelf-life of each Product as specified in the Seller's catalogue and, in the case of a specific order, according to such specific order.
- 11.b All disputes differences or questions at any time arising between the parties as to the construction, validity and performance of these terms and conditions of sale or to sales hereunder or as to any matter or thing arising there of or in any way connected therewith shall be referred to the arbitration of a single arbitrator in Israel. The arbitrator shall be appointed at the request of either party by the Chairman, of the time being, of the Manufacturers Association of Israel.
- 11.c If any such disputes, differences or questions not be submitted to arbitration and decided by an arbitrator as aforesaid, the exclusive jurisdiction to deal therewith shall be vested in the Courts of Tel Aviv, Israel
- 11.d These terms and conditions shall be subject to and construed in accordance with Israel law.
- 11.e If Buyer is in breach of its obligations under this contractor sale. Buyer shall remain liable for all unpaid charges and sums due to Seller and shall reimburse Seller for all damages suffered or incurred by Seller as a result of Buyer's breach. The remedies provided herein shall be in addition to all other legal means and remedies available to Seller under these terms and conditions of sale and under law.



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