7. PCR Essentials

DNA Polymerases

Other Polymerases

Other enzymes

Nucleotides

DNA Ladders

Photo: Tissue in the microscopic view

DNA Polymerases

DNA Polymerases Selection Guide:

	Catalog Number	Page	5'→3´ exonuclease	3'→5´ exonuclease	Target length	Difficult template	MasterMix	Blunt or 3´-A ends	Fidelity vs Taq	Primary applications
Horse-Power™ Taq-DNA Polymerase	P0023-S P0023 P0024 P0020 P0025 P0019 P0026 P0035	103	+	-	≤ 5kb	✓	✓	3´-A	1x	· Routine PCR.
HotBegan™ Hot Start Taq-DNA Polymerase	P0028 P0030	104	+	-	≤ 5kb	✓	✓	3´-A	1x	· Hot Start and real time PCR.
FastPangea™ High Fidelity DNA polymerase	P0031 P0032 P0033 P0061	105	-	+++	≤ 20kb	✓	✓	Blunt	50x	· Ultra High Fidelity PCR.
FastPangea™ Long PCR DNA polymerase	P0060 P0022	106	+	++	≤ 15kb	✓	✓	3´-A/Blunt	6.5x	· High Fidelity PCR.
Horse-Power™ Green-Taq DNA Polymerase	P0029	107	+	-	≤ 5kb	✓	✓	3´-A	1x	· Routine PCR.
Horse-Power™ Red-Taq DNA Polymerase	P0027	107	+	-	≤ 5kb	✓	✓	3´-A	1x	· Routine PCR.
SNP Taq DNA Polymerase	P0055 P0056	110	-	-	≤ 5kb		✓	3´-A	1x	· High specific PCR. · Multiplex PCR. · Real-Time PCR with intercalation dyes. · Mini-Sequencing, SNP-genotyping.
Bst DNA Polymerase	P0045 P0046	109	+	-	Whole genome	✓		Blunt		· Isothermal amplification. · Whole genome amplification. · Multiple displacement amplification. · Sequencing DNA with high GC content and secondary structures. · Rapid sequencing from nanogram amounts of DNA Template.
DNA Polymerase I	P0040	108	+	++		✓		Blunt		· Nick translation. · Removal of 3' protruding DNA ends (without dNTPs).
DNA Polymerase I, Large (Klenow) Fragment	P0041	108	-	++				Blunt		· DNA replication when exonuclease activity in 3' needs to be avoided (fill in large gaps).
T4 DNA Polymerase	P0042 P0043	111	-	+++				Blunt		· DNA replication when exonuclease activity. · Amplification of large DNA fragments. · Preparation of radioactive probes. ·In 3' needs to be avoided (fill in larges gaps):

Standard and High Throughput PCR

Horse-Power™ Taq DNA Polymerase, Recombinant

Highly purified for routine amplifications

Ordering info:

Concentration: 5 U/µL	
Cat No.	Size
P0023-S	200 U
P0023	500 U
P0024	1,000 U
P0020	10,000 U

Includes for 500 U:
- 100 µL Horse-Power™ Taq DNA Polymerase (5 U/µL)
- 25 mM $MgCl_2$ (1.5 mL)
- 1.5 mL Buffer (10x)

Concentration: 1 U/µL	
Cat No.	Size
P0025	500 U
P0019	5,000 U

Includes for 500 U:
- 500 µL Horse-Power™ Taq DNA Polymerase (1 U/µL)
- 25 mM $MgCl_2$ (1.5 mL)
- 1.5 mL Buffer (10x)

With dNTPs	
Cat No.	Size
P0026	500 U+ 2 mM each (1 mL)

Includes for 500 U:
- 100 µL Horse-Power™ Taq DNA Polymerase (5 U/µL)
- 25 mM $MgCl_2$ (1.5 mL)
- 1.5 mL Buffer (10x)
- 1 mL TruePure™ dNTPs (2 mM each)

MasterMix (2x)	
Cat No.	Size
P0035	2 x 1.25 mL (2x)

(2.5 mL= 250 rxn)

Includes for 2.5 mL:
- 2 x 1.25 mL Horse-Power™ Taq DNA Polymerase MasterMix (2x)

Related products:
- TruePure™ dNTPs (p.115)
- Loading Buffers (p.117)
- TAE (p.137)
- BrightMAX™ DNA Ladders (p.116)
- pSpark® TA DNA Cloning vectors (p.16)

Description:
Horse-Power™ Taq DNA Polymerase is pure, versatile and thermostable recombinant enzyme produced in an *E. coli* strain, which carries the cloned pol gene from *Thermus aquaticus*. The enzyme has 5'→3' polymerase activity and a weak 5'→3' exonuclease activity but no 3'→5' exonuclease activity (proofreading).

Advantages & Features:
- **Highest purity:** > 98% confirmed by SDS-PAGE.
- **Highest quality:** high activity, specificity, thermostability and performance in PCR.
- **Highly efficient:** reactivation buffer improved.
- **Thermostable:** half-life at 94° C is 40 minutes.
- **Adds extra nucleotides:** preferentially adenine, without template at 3´ends leaving 3´overhangs PCR fragments.
- **Incorporates modified nucleotides:** biotinylated, fluorescently labelled, etc.
- **Molecular Weight:** 94 kDa.
- **Convenient:** available in different concentrations, sizes and solutions.
- **Complete solution:** includes $MgCl_2$.

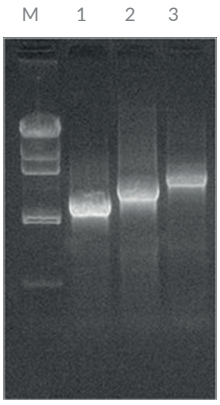
Assay conditions:
25 mM Tris-HCl pH 9.0 at 25 °C, 50 mM KCl, 2 mM $MgCl_2$, 0.1 mg/mL gelatine, 200 µM dATP, dGTP, dTTP, 100 µM [α32-P] dCTP (0.05 µCi/nmol) and 12.5 µg activated salmon sperm DNA.

Unit definition:
One unit is defined as the amount of enzyme required to catalyse the incorporation of 10 nanomoles of dNTPs into acid-insoluble material in 30 minutes at 74 °C.

Applications:
- Routine amplifications.
- Colony screening (see Horse-Power™ Red-Taq DNA Polymerase, p.107).
- Amplifications up to 5 kb using plasmid, viral or genomic DNA as template.
- PCR fragments amplification for TA or GC cloning.

Quality control:
- Functionally tested in PCR.
- Free of bacterial DNA (by qPCR).
- Exempt of nucleases (endo, exo and ribonucleases) activities guaranteed by appropriate quality tests.

Figure 7.1.: Amplification of different length fragments in 25 cycles of PCR.



M λ *HindIII*
1 3 kb
2 4 kb
3 5 kb

Agarose 0.7% in TAE 1X stained with Gelgreen. Lane 1-2 were loaded with 5 µL of PCR while lane 3 was loaded with 10 µL.

HotBegan™ Hot Start DNA Polymerase

For a specific, efficient and reliable amplifications of DNA up 160 fg

Ordering info:

Concentration: 5 U/µL	
Cat No.	Size
P0028	500 U

Includes for 500 U:

- 100 µL HotBegan™ Hot Start DNA Pol. (5 U/µL)
- 25 mM $MgCl_2$ (1.5 mL)
- 1.5 mL Buffer B (10x)

MasterMix (2x)	
Cat No.	Size
P0030	2 x 1.25 mL (2x)

(2.5 mL=250 rxn)

Includes for 2.5 mL:

- 2 x 1.25 mL HotBegan™ Hot Start DNA Polymerase MasterMix (2x)

Description:

HotBegan™ Hot Start Taq DNA Polymerase is a specific, efficient and sensitive Hot Start DNA Polymerase designed to minimize unspecific amplification improving PCR specificity. It is a Horse-Power™ Taq DNA Polymerase bound to a proprietary antibody that blocks polymerase activity until denaturation step occurs. The heat labile antibodies are rapidly inactivated by raising the temperature (4 minutes at 95-97°C). This prevents or minimizes primer-dimer and non-specific products.

Like Horse-Power™ Taq DNA Polymerase, the enzyme has 5'→3' polymerase activity and a weak 5'→3' exonuclease activity but no 3'→5' exonuclease activity (proofreading). Before enzyme activation none of enzyme activities are detectable.

Advantages & Features:

- ✓ **High specificity:** minimize unspecific amplification.
- ✓ **Efficient:** prevents or minimizes primer-dimer and nonspecific products.
- ✓ **Great sensitivity:** amplifies from a femptograms of DNA targets.
- ✓ **Inactive:** at Room Temperature.
- ✓ **Optimized:** adds extra nucleotides (preferentially adenine) without template at 3´ends leaving 3´overhangs PCR fragments.
- ✓ **Powerful:** amplification of targets up to 5 kb.
- ✓ **Convenient:** available in kit and Master Mix solution.

Applications:

- ✓ qPCR.
- ✓ RT-PCR and RT-PCR.
- ✓ Genotyping with Taqman probes.
- ✓ PCR fragments amplification for TA or GC cloning.
- ✓ Amplification from a limited DNA template or low copy number genes.

Quality control:

- ✓ Functionally tested in qPCR.
- ✓ None detected bacterial DNA (by qPCR).
- ✓ Exempt of nucleases (endo, exo and ribonucleases) activity guaranteed by appropriate quality tests.

Unit definition:

One unit is defined as the amount of enzyme required to catalyse the incorporation of 10 nanomoles of dNTPs into acid-insoluble material in 30 minutes at 74°C.

Figure 7.2.: Amplification of up 160 fg.

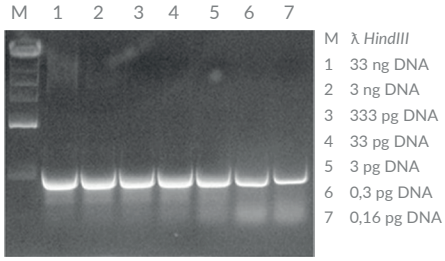
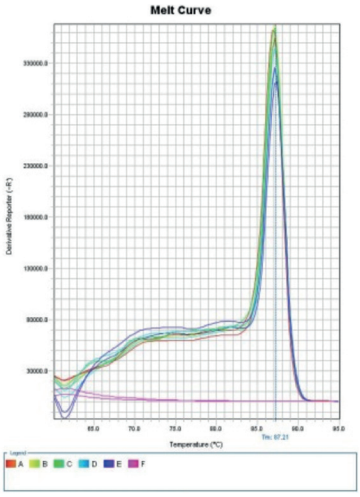
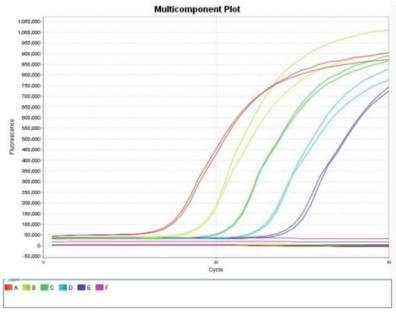


Figure 7.3.: Actin Melt Curve.



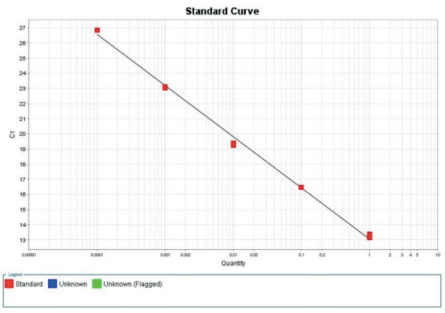
Tm= 93.03° C

Figure 7.4.: qPCR curve from actin amplification from cDNA of HEK-293 cells.



A= 2 * 10^{-1}	m= -3.374
B= 2 * 10^{-2}	y= 13.045
C= 2 * 10^{-3}	R^2= 0.996
D= 2 * 10^{-4}	Efficiency %= 97.884
E= 2 * 10^{-5}	
F= NTC	

Figure 7.5.: Actin Standard Curve.



FastPANGEA™ High Fidelity DNA Polymerase

For robust, fast and extreme fidelity for all PCR applications

Ordering info:

Concentration: 2 U/µL	
Cat No.	Size
P0031	50 U
P0032	100 U
P0033	500 U

Includes for 100 U:

- 100 U FastPANGEA™ High Fidelity DNA Pol. (2U/µl)
- 500 µL Buffer Uni (2.5x)
- 100 µL DMSO
- 100 µL $MgCl_2$ (25 mM)

MasterMix (2x)	
Cat No.	Size
P0061	200 rxn

Includes for 200 rxn:

- 2 x 1 mL FastPANGEA™ High Fidelity DNA Pol. (2x)
- 50 µL DMSO (100%)
- 100 µL $MgCl_2$ (25 mM)

Related products:

- pSpark® DNA cloning vectors (p.12)
- TruePure™ dNTPs (p.115)
- TAE (p.137)
- TBE (p.137)
- Custom solutions (p.147)
- BrightMAX™ DNA Ladders (p.116)
- Loading Buffers (p.117)

Description:

FastPANGEA™ High Fidelity DNA Polymerase is a second generation High-fidelity DNA Polymerase that offers extreme performance for all PCR applications. It generates long templates with an accuracy and speed previously unattainable with other thermostable DNA polymerases. The error rate of FastPANGEA™ DNA polymerase is at least 50-fold lower than a normal Taq DNA Polymerase.

It possesses the 5'→3´ DNA polymerase activity, 3´→5´ exonuclease activity and it generates PCR products with blunt ends. It is also suitable for amplification of long amplicons such as 10-15 kb of genomic DNA.

Advantages & Features:

- ✓ **Second generation** High-Fidelity DNA polymerase.
- ✓ **Extreme Fidelity:** error rate is > 50x more accurate than normal Taq DNA Polymerase.
- ✓ **Robust:** maximal success with minimal optimisation.
- ✓ **Very Fast:** extension times are 15-30 seconds/kb.
- ✓ **Increased product yield:** with minimal amount of enzyme.
- ✓ **Versatile:** ideal for routine PCR, as well as long or difficult templates.

Unit definition:

One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTPs into acid-insoluble form in 30 minutes at 75°C under assay conditions: 25 mM TAPS-HCl, pH 9.0 (at 25°C), 100 mM KCl, 1.5 mM $MgCl_2$, 1 mM Beta-mercaptoethanol, 200 µM each dNTP and 10 µg activated calf thymus DNA in 50 µl.

Applications:

- ✓ PCR-Cloning.
- ✓ Primers extension.
- ✓ Long or difficult amplification.
- ✓ High-Throughput PCR.

Quality control:

- ✓ Functionally tested in PCR.
- ✓ Undetected bacterial DNA (by PCR).

Note:
Highly recommended for cloning into **pSpark® DNA** cloning vectors.

FastPANGEA™ Long PCR DNA Polymerase

For an accurate and robust PCR amplification of fragments up to 15 kb

Ordering info:

Concentration: 5 U/µL	
Cat No.	Size
P0060	200 U

Includes:

- 200 U FastPANGEA™ Long PCR DNA Pol. (5U/µl)
- 1.5 mL Buffer (10x)
- 1.5 mL $MgCl_2$ (25 mM)
- 50 µL DMSO (100%)

MasterMix (2x)	
Cat No.	Size
P0022	200 rxn

Includes for 200 rxn:

- 2 x 1 mL FastPANGEA™ Long PCR DNA Polymerase MasterMix (2x)
- 50 µL DMSO (100%)
- 100 µL $MgCl_2$ (25 mM)

Related products:

- pSpark® DNA Cloning vectors (p.12)
- TruePure™ dNTPs (p.115)
- TAE (p.137)
- TBE (p.137)
- BrightMAX™ DNA Ladders (p.116)
- Loading Buffers (p.117)

Description:

FastPANGEA™ Long PCR DNA Polymerase is an accurate and robust enzyme that combines Horse-Power™ Taq DNA Polymerase and a DNA proofreading Polymerase with 3' to 5' exonuclease activity that is optimized for PCR amplification of very long DNA templates (long range PCR).

As it is already well-known, Taq DNA Polymerase is inefficient at amplifying fragments larger than 3–5 kb due to its inability to repair nucleotide mismatches following misincorporation. The addition of a small quantity of proofreading enzyme allows mismatches to be repaired and extension to continue, resulting in the amplification of long amplicons with high yield. The presence of the proofreading polymerase significantly increases fidelity (6.5x) as compared to Taq DNA Polymerase alone. This mixture of enzymes allows for long and accurate PCR amplification of targets from a variety of templates, such as 5-15 kb of genomic DNA.

It generates long templates with an accuracy and speed previously unattainable with other thermostable DNA polymerases. As well, it possesses 3´→ 5´ exonuclease activity and it generates PCR products with blunt ends and generate 3´-adenine overhang in amplified DNA and thus such Taq amplified DNA could be cloned into T-vectors.

Advantages & Features:

- **High Fidelity:** 6.5 times higher fidelity than Taq DNA Polymerase alone.
- **Robust:** PCR amplification of fragments up to 15 kb such as 5-15 kb of genomic DNA.
- **High Yield:** increased amplicon specificity and yield.
- **Accurate.**
- **Versatile:** proven performance for long or difficult templates.

Applications:

- PCR-Cloning.
- Primers extension.
- Long or difficult amplification.
- High-Throughput PCR.

Quality control:

- Functionally tested in PCR.
- Free of bacterial DNA (by qPCR).

Unit definition:

One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTP's into acid-insoluble form in 30 minutes at 75 °C under assay conditions: 25 mM TAPS-HCl, pH 9.0 (at 25 °C), 100 mM KCl, 1.5 mM $MgCl_2$, 1 mM Beta-mercaptoethanol, 200 µM each dNTP and 10 µg activated calf thymus DNA in 50 µl.

Note:
Highly recommended for cloning with **pSpark® DNA** cloning vectors.

Horse-Power™ Green-Taq DNA Polymerase

For an optimized, accurate and fast visual tracking of DNA migration

Ordering info:

Cat No.	Size
P0029	5 x 100 rxn

Includes for 500 rxn:

· 5 x 100 rxn Horse-Power™ Green-Taq DNA Polymerase (2x)

Related products:

· pSpark® DNA Cloning vectors (p.12)
· TruePure™ dNTPs (p.115)
· TAE (p.137)
· TBE (p.137)
· BrightMAX™ DNA Ladders (p.116)
· Loading Buffers (p.117)

Description:

Horse-Power™ Green-Taq DNA Polymerase is an optimized, accurate and ready-to-use (2x) MasterMix that incorporates all PCR reaction components: TruePure™ dNTPs, PCR buffer, Mg^{2+} and Horse-Power™ Taq DNA Polymerase.

The mix also incorporates an agarose Loading Buffer including two tracking dyes (blue and yellow dye) for visual tracking of DNA migration and a dense compound to facilitate the drop-down of the samples into the well agarose gels. The migration in 1% agarose gel of blue dye is 3 to 5 kb DNA fragments, meanwhile yellow dye migrates in 1% agarose gel faster than 10 bp DNA fragments.

Advantages & Features:

- ✔ **Optimized:** adds extra nucleotides (preferentially adenine) without template at 3´ends leaving 3´overhangs PCR fragments.
- ✔ **Time-saving protocol:** ready-to-use format that saves time in the PCR process and in agarose loading samples.
- ✔ **Complete solution:** includes all PCR reaction components except primers and template.

Assay conditions:

Enzyme activity is assayed in the following mixture: 25 mM Tris-HCl pH 9.0 at 25°C, 50 mM KCl, 2 mM $MgCl_2$, 0.1 mg/mL gelatine, 200 µM Φ dATP, dGTP, dTTP, 100 µM [α32-P] dCTP (0.05 µCi/nmol) and 12.5 µg activated salmon sperm DNA.

Applications:

- ✔ Design for medium or high throughput applications (e.g. colony screening).
- ✔ PCR fragments amplification for TA or GC cloning.

Quality control:

- ✔ Functionally tested in PCR.
- ✔ Free of bacterial DNA (by PCR).
- ✔ Exempt of nucleases (endo, exo and ribonucleases) activities guaranteed by appropriate quality tests.

Concentration:

2x (Buffer Green 2X; TruePure™ dNTPs 0.4 mM each; Horse-Power™ Taq DNA Polymerase 0.2 U/µL, Glycerol 24%).

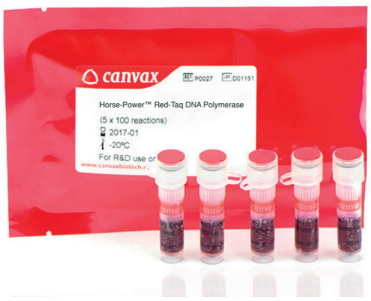
Green dye Agarose Mobility*:

Agarose Gel Concentration (%)	Blue Dye (bp)	Yellow Dye (bp)
0.5 - 1.5	10,000-4,000	<20
2.0 - 3.0	750-200	<20

*in TAE Buffer

Horse-Power™ Red-Taq DNA Polymerase

For an optimized, accurate and fast visual tracking of DNA migration



Ordering info:

Cat No.	Size
P0027	5 x 100 rxn

Includes for 500 rxn:

· 5 x 100 rxn Horse-Power™ Red-Taq DNA Polymerase MasterMix (2.5x)

Related products:

· TruePure™ dNTPs (p.115)
· Loading Buffers (p.117)
· TAE (p.137)
· TBE (p.137)
· Custom solutions (p.147)
· BrightMAX™ DNA Ladders (p.116)
· pSpark® TA DNA Cloning vectors (p.16)

Description:

Horse-Power™ Red-Taq DNA Polymerase is an optimized, accurate and ready-to-use (2,5x) MasterMix that incorporates all PCR reaction components: TruePure™ dNTPs, PCR buffer, Mg^{2+} and Horse-Power™ Taq DNA Polymerase.

The mix also incorporates an agarose Loading Buffer including a red dye for visual tracking of DNA migration and a dense compound to facilitate the drop-down of the samples into the well agarose gels.

Advantages & Features:

- ✔ **Optimized:** adds extra nucleotides (preferentially adenine) without template at 3´ends leaving 3´overhangs PCR fragments.
- ✔ **Time-saving:** ready-to-use format that saves time in the PCR process and in agarose loading samples.
- ✔ **Complete solution:** includes all PCR reaction components except primers and template.

Concentration:

2.5x (Buffer Red 2.5X, TruePure™ dNTPs 0.5 mM each, HorsePower™ Taq DNA Polymerase 0.25 U/µL, Glycerol 30%).

Assay conditions:

25 mM Tris-HCl pH9.0 at 25 °C, 50 mM KCl, 2 mM $MgCl_2$, 0.1 mg/mL gelatine, 200 µM dATP, dGTP, dTTP, 100 µM [α32-P] αCTP (0.05 µCi/nmol) and 12.5 µg activated salmon sperm DNA.

Applications:

- ✔ Design for medium or high throughput applications (e.g. colony screening).
- ✔ PCR fragments amplification for TA or GC cloning.

Quality control:

- ✔ Functionally tested in PCR.
- ✔ Free of bacterial DNA (by PCR).
- ✔ Exempt of nucleases (endo, exo and ribonucleases) activities guaranteed by appropriate quality tests.

Red dye Agarose Mobility*:

Agarose Gel Concentration (%)	Migration Rate (bp)
0.7	3,000
1.0	1,500
1.5	900
2.0	300
3.0	>100

*in TAE Buffer

Related Polymerases

DNA Polymerase I *(E. coli)*

Ordering info:

Concentration: 10 U/µL	
Cat No.	Size
P0040	500 U

Includes for 500 U:

· 50 µL DNA Polymerase I (10 U/µL)
· 1 mL Reaction Buffer (10x)

Related products:

· TruePure™ dNTPs (p.115)
· DNA Polymerase I, Large (Klenow) Fragment (p.108)
· BrightMAX™ DNA Ladders (p.116)

Description:

DNA Polymerase I is a multifunctional enzyme that combines a DNA Polymerase activity, a 5´→ 3´exonuclease activity and a 3'→5' proofreading exonuclease activity. The 5´→3´ exonuclease activity enables the enzyme to use nicks and gaps in the DNA as starting points for labelling DNA by nick translation.

Advantages & Features:

- ✓ **Native:** isolated from *E. coli* cells with a cloned fragment of the *polA* gene.
- ✓ **DNase I-dependent nick translation:** second-strand synthesis in cDNA cloning, fill-in of 5´ overhangs.
- ✓ **Complete solution:** supplied with 10x Reaction Buffer.

Assays conditions:

67 mM potassium phosphate (pH 7.4), 6.7 mM $MgCl_2$, 1 mM 2-mercaptoethanol, 0.033 mM dATP, 0.033 mM dTTP, 0.4 MBq/mL [3H]-dTTP and 62.5 µg/mL poly(dA-dT)·poly(dA-dT).

Applications:

- ✓ High percentage incorporation of radioactivity for nick translation assays.
- ✓ Manufacturing of alternating copolymers such as poly d(A-T) and homopolymers such as poly dG-poly dC.
- ✓ Klenow fragment: DNA sequencing, fill-in of 5'overhangs and removal of 3' overhangs to form blunt ends and second strand synthesis in mutagenesis.

Quality control:

- ✓ Absence of covalently conversion of closed circular DNA to nicked DNA after incubation of 20 units of DNA polymerase I with 1 µg of pUC18 DNA for 4 hours at 37°C.

DNA Polymerase I, Large (Klenow) Fragment

Ordering info:

Concentration: 10 U/µL	
Cat No.	Size
P0041	500 U

Includes for 500 U:

· 50 µL DNA Polymerase I (10 U/µL)
· 1 mL Reaction Buffer (10x)

Related products:

· TruePure™ dNTPs (p.115)
· Custom solutions (p.147)
· BrightMAX™ DNA Ladders (p.116)

Description:

Klenow Fragment is the Large Fragment of DNA Polymerase I. It shows 5'→3' polymerase activity and 3'→5' exonuclease (proofreading) activity, but lacks 5'→3' exonuclease activity of DNA Polymerase I.

Advantages & Features:

- ✓ **Recombinant:** isolated from *E. coli* cells with a cloned fragment of the *polA* gene.
- ✓ **DNase I-dependent nick translation:** second-strand synthesis in cDNA cloning, fill-in of 5´overhangs.
- ✓ **Generates probes** using random primers.
- ✓ **Creates blunt ends.**
- ✓ **Complete solution:** supplied with 10x Reaction Buffer.

Unit definition:

One unit of the enzyme catalyses the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction in 30 min at 37°C, using poly(dA-dT)·poly(dAdT) as a template primer.

Applications:

- ✓ DNA sequencing, fill-in of 5' overhangs and removal of 3' overhangs to form blunt ends and second strand synthesis in mutagenesis.
- ✓ DNA blunting by fill-in of 5'-overhangs or removal of 3'-overhangs.
- ✓ Random-primed DNA labeling.
- ✓ Labeling by fill-in 5'-overhangs of dsDNA.
- ✓ DNA sequencing by the Sanger method.
- ✓ Site-specific mutagenesis of DNA with synthetic oligonucleotides.
- ✓ Second strand synthesis of cDNA.

Quality control:

- ✓ Absence of covalently conversion of closed circular DNA to nicked DNA after incubation of 20 units of DNA polymerase I with 1 µg of pUC18 DNA for 4 hours at 37°C.

T4 DNA Polymerase

Ordering info:

Concentration: 5 U/µL	
Cat No.	Size
P0042	100 U
P0043	500 U

Includes for 100 U:

· 20 µL T4 DNA Polymerase (5 U/µL)
· 1 mL Reaction Buffer (5x)

Related products:

· DNAse (p.112)
· TruePure™ dNTPs (p.115)
· pSpark® DNA cloning vectors (p.12)
· Custom solutions (p.147)

Description:

Bacteriophage **T4 DNA Polymerase** is a DNA-directed 5' to 3' DNA polymerase. It is the product of gene 43 from bacteriophage T4 and is therefore often referred to as T4 gp43 DNA Polymerase. The enzyme catalyzes the polymerization of deoxynucleotide triphosphates in a 5' to 3' direction. It possesses very active 3' to 5' exonuclease activity that is more active on single than double stranded DNA, T4 DNA polymerase has no 5' to 3' exonuclease activity. For polymerase activity the enzyme requires DNA with a 5' protruding end and a high concentration of TruePure™ dNTPs.

Advantages & Features:

- ✔ **Recombinant:** isolated from a recombinant source (*E. coli* cells with a cloned gene 43 from bacteriophage T4).
- ✔ **Powerful:** stronger 3'-5' exonuclease activity on single-stranded than on double-stranded DNA.
- ✔ **Complete solution:** supplied with 10x Reaction Buffer.

Unit Definition:

One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 minutes at 37°C using DNAse I-Nicked DNA as template-primer.

Applications:

- ✔ Generation of blunt double-stranded DNA from DNA containing 5' overhangs.
- ✔ Generation of blunt double-stranded DNA from DNA containing 3' overhangs.
- ✔ 5'-end or 3'-end labeling of double-stranded DNA
- ✔ *In vitro* mutagenesis.

Quality control:

- ✔ Absence of covalently conversion of closed circular DNA to nicked DNA after incubation of 20 units of DNA polymerase I with 1 µg of pUC18 DNA for 4 hours at 37°C.

Bst DNA Polymerase, Exonuclease Minus

Ordering info:

Concentration: 8 U/µL	
Cat No.	Size
P0045	2,000 U
P0046	10,000 U

Includes for 2,000 U:

· Bst DNA Polymerase (8 U/µL)
· Reaction Buffer (10x)
· 100mM $MgSO_4$ solution

Related products:

· TruePure™ dNTPs (p.115)
· Custom solutions (p.147)

Description:

Large Fragment of **Bst DNA Polymerase, Exonuclease Minus**, is isolated from a *Bacillus stearothermophilus*.

It catalyzes 5'→3' synthesis of DNA and lacks 5' →3' and 3' → 5' exonuclease activities.

Advantages & Features:

- ✔ **High Purity:** >99% by SDS PAGE.
- ✔ **Reverse transcription activity.**
- ✔ **Optimized:** Increased activity.
- ✔ **Strong strand displacement activity.**

Unit definition:

One unit catalyzes the incorporation of 10 nmol of dNTP into acid-insoluble material in 30 minutes at 65 °C in 20 mMTris-HCl pH 8.8, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, 0.1% Triton X-100, 30 nM M13mp18 ssDNA, 70 nM M13 sequencing primer(-47) 24-mer 200 µM dGTP, dATP, dTTP, dCTP (a mix of unlabeled and [^{32}P]dCTP) and 0.1 mg/mL BSA.

Applications:

- ✔ Nucleic acid amplification methods.
- ✔ Whole genome amplification.
- ✔ Multiple displacement amplification.
- ✔ DNA Sequencing.

Quality control:

- ✔ Purity: >99% by SDS PAGE.
- ✔ Exempt of nucleases (endo, exo and ribonucleases) activities guaranteed by appropriate quality tests.
- ✔ 10 µl of the enzyme was tested for *E. coli* genomic DNA contamination by PCR amplifying with the *E. coli* 16S ribosomal primers.

SNP Taq DNA Polymerase

Ordering info:

Concentration: 20 U/µL	
Cat No.	Size
P0055	500 U
P0056	2,500 U

Includes for 500 U:

- SNP Taq DNA Polymerase (20 U/ µL)
- Reaction Buffer (5x) without $MgCl_2$
- 100mM $MgCl_2$

Description:

SNP Taq DNA Polymerase is an efficient, High Fidelity and specific Hot-Start Polymerase with special N-terminal deletion and proprietary amino acids substitutions introduced into the active domine of the enzyme. Due this special modification the enzyme increases its sensitivity to mismatches at 3'-end of the primer. For this reason, unspecific amplicons are formatted due the non-perfect primers annealing.

Advantages & Features:

- **Efficient:** 10 to 15-fold lower mutation rate than normal Taq DNA Polymerase.
- **High Versatility:** allele-specific amplification of DNA fragments.
- **High Specificity:** lowest background AS-PEX and AS-PCR.
- **Cost avoidance:** reduce the use of expensive primer dimers.

Unit definition:

One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTPs into acid-insoluble form in 30 minutes at 72°C.

Applications:

- High specific or Multiplex PCR.
- Real-Time PCR with intercalation dyes.
- High Fidelity dNTPs and ddNTPs.
- Mini Sequencing procedures.
- Allele-specific primer extension (AS-PEX).
- SNP genotyping by allele-specific PCR (AS-PCR).
- Single Nucleotide Polymorphism (SNP).

Quality control:

- Functionally tested in PCR.
- Free of bacterial DNA (by PCR).
- Exempt of nucleases (endo, exo and ribonucleases) activities guaranteed by appropriate quality tests.

Related products:

- TruePure™ dNTPs (p.115)
- BrightMAX™ DNA Ladders (p.116)
- Custom solutions (p.147)

AMV Reverse Transcriptase

Ordering info:

Cat No.	Size
P0070	300 U
P0071	1,000 U

Includes:

- AMV Reverse Transcriptase (10U/µL)
- Reaction Buffer (5x)

Description:

AMV Reverse Transcriptase, encoded by Avian Myeloblastosis Virus (AMLV) is an RNA dependent DNA polymerase that synthesizes the complementary cDNA first strand from a single-stranded RNA template. AMV Reverse Transcriptase (AMV RT) catalyzes the polymerization of DNA using template DNA, RNA or RNA:DNA hybrids.

Applications:

- RT PCR.
- Synthesis of cDNA.
- RNA Sequencing.

Quality control:

- Exempt of nucleases (endo, exo and ribonucleases) activities.
- Purity: >90% as judged by SDS-polyacrylamide gels with blue staining.

MMLV Reverse Transcriptase

Ordering info:

Cat No.	Size
P0073	10,000 units
P0074	5 x 10,000 units

Includes:

- MMLV Reverse Transcriptase (200U/µL)
- Reaction Buffer (5x)

Description:

MMLV Reverse Transcriptase (MMLV-RT), encoded by Moloney Murine Leukemia Virus (MMLV) is an RNA-dependent DNA polymerase that synthesizes the cDNA first strand from a single-stranded RNA template to which a primer has been hybridized. MMLV-RT will also extend primers hybridized to single-stranded DNA.

Applications:

- RT PCR.
- Synthesis of cDNA.
- mRNA 5'-end Mapping by Primer Extension Analysis.
- End-labeling of DNA.
- Dideoxynucleotide Sequencing.

Quality control:

- Exempt of nucleases (endo, exo and ribonucleases) activities.
- Purity: >90% as judged by SDS-polyacrylamide gels with blue staining.

Related enzymes

T4 DNA Ligase

For highly efficient, accurate and easy ligation of DNA insert into plasmid vectors in 5-15 minutes

Ordering info:

Concentration: 5 U/µL	
Cat No.	Size
C005	300 U
C006	1,000 U

Includes for 300 U:

· 60 µL T4 DNA Ligase (5U Weiss/µL)
· 250 µL T4 DNA Ligase Buffer (5x)

Related products:

· FastPANGEA™ High Fidelity DNA Polymerase (p.105)
· pSpark® DNA cloning vectors (p.12)
· CVX5α™ Chemically Competent cells (p.18)
· Horse-Power™ Taq DNA Polymerase (p.103)
· Custom solutions (p.147)

Description:

T4 DNA Ligase is highly efficient, accurate and rapid enzyme designed for an efficient ligation of cohesive and blunt ended DNA insert into plasmid vectors in 5-15 minutes. It is based on the combination of T4 DNA Ligase with a premium 5x Ligation Buffer. The ligation reaction mixture is used directly for bacterial transformation using conventional transformation procedures. It enables sticky end or blunt end DNA ligation in only 5 minutes at Room Temperature. The efficiency of this fast ligation is 24% at 5 minutes and 75% at 15 minutes.

Advantages & Features:

- ✓ **Recombinant:** isolated from *E. coli*.
- ✓ **Highly efficient:** 3,500 white colonies and 10 blue colonies expected under optimal conditions.
- ✓ **Fast and easy protocol:** efficient ligation in only 5 minutes.
- ✓ **Powerful:** allows cloning insert amounts < 1 ng/kb.

Unit Definition:

One Weiss unit of the enzyme catalyses the conversion of 1 nmol of [^{32}PPi] into Norit- absorbable form in 20 min at 37° C. One Weiss unit is equivalent to approximately 200 cohesive end ligation (CEL) units and one CEL unit is defined as the amount of enzyme required to give 50% ligation of HindIII fragments of 1 µg lambda DNA in 30 min. at 16° C.

Applications:

- ✓ Routine cloning procedure: blunt and cohesive double stranded DNA cloning.
- ✓ TA cloning.
- ✓ Joining of adaptors with blunt or cohesive ends.

Quality control:

- ✓ Functionally tested in the ligation of a blunted fragment of 1 kb into pSpark® I vector.
- ✓ Exempt of nucleases (endo, exo and ribonucleases) and phosphatases activity guaranteed by appropriate quality tests.

Note:
One Weiss unit is equivalent to approximately 200 CEL units.

RNase A (Ribonuclease A)

Ordering info:

Cat No.	Size
EZ0002	1 mL
EZ0003	5 x 1 mL

Includes for 1 mL:

· 1 mL RNase A Solution (10 mg/mL)

Related products:

· WideUse™ Plasmid Purification Kit (p.92)
· HigherPurity™ Soil DNA Isolation Kit (p.90)
· MagBeads™ Bacteria G (-) Genomic DNA Isolation (p.94)
· HigherPurity™ Yeast Genomic DNA Isolation Kit (p.89)
· HigherPurity™ Plant DNA Purification Kit (p.88)

Description:

RNase A (ribonuclease A) is a bovine pancreatic endoribonuclease that cleaves single-stranded RNA. It catalyzes the cleavage of the phosphodiester bond between the 5'-ribose of a nucleotide and the phosphate group attached to the 3'-ribose of an adjacent pyrimidine nucleotide. This cleavage forms a 2', 3'-cyclic phosphate, which is then hydrolysed to the corresponding 3'-nucleoside phosphate.

Advantages & Features:

- ✓ **Small single-chain polypeptide:** 124 residues, ~13.7 kDa.
- ✓ **Avoids the use of co-factors and divalent cations.**
- ✓ **High specific activity:** > 100 Kunitz units per mg protein.
- ✓ **High Quality:** free of DNase, Proteinase or exonuclease contamination.
- ✓ **Time-saving protocol:** avoids to RNAse heat step before use.

Unit definition:

One unit of the enzyme causes an increase in absorbance of 1.0 at 260 nm when yeast RNA is hydrolyzed at 37 °C and pH 5.0. 50 units are approximately equivalent to 1 Kunitz unit.

Applications:

- ✓ RNA removal during DNA isolation.
- ✓ RNA sequence analysis.
- ✓ RNase protection assays.
- ✓ RNA quantification or mapping.
- ✓ Purifying plasmid DNA.
- ✓ Genomic DNA isolation.
- ✓ Molecular weight marker.

Quality control:

- ✓ Functionally tested for RNA degradation in a plasmid DNA purification protocol.
- ✓ Exempt of contaminant exo- and endodeoxyribonuclease activities guaranteed by appropriate quality tests.

Note:
One Kunitz unit equals to 50 Worthington units.

DNase I

Ordering info:

Cat No.	Size
EZ0018	1 mL
EZ0019	5 x 1 mL

Includes for 1 mL:

· 1 mL DNase I (10 mg/mL)
· 1 mL Reaction Buffer (10x)

Related products:

· HigherPurity™ Tissue Total RNA Purification kit (p.98)
· HigherPurity™ Plant RNA Purifiction kit (p.97)
· HigherPurity™ Blood / Cultured Cell Total RNA kit (p.98)

Description:

DNase I is a recombinant endonuclease that cleaves DNA preferentially at phosphodiester linkages adjacent to a pyrimidine nucleotide, to release di-, tri- and oligonucleotide products (on average producing tetranucleotides) with 5'-phosphorylated and 3'-hydroxylated ends.

It acts on single-stranded DNA, double-stranded DNA, RNA-DNA hybrids and chromatin. DNase I requires bivalent cations (Mg^{2+} and Ca^{2+}) for maximal activity.

Advantages & Features:

- ✓ **Recombinant:** bovine Pancreatic, purified from *E. coli* (29 kDa monomer).
- ✓ **High specific activity:** > 2,000 Kunitz units per mg protein.
- ✓ **High Quality:** free of RNase and Proteinase contamination.
- ✓ **Complete solution:** supplied with 10x Reaction Buffer.

Unit definition:

One Kunitz unit is defined as the amount of enzyme required for the complete degradation of 1 µg of plasmid DNA in 10 minutes at 37˚C.

Applications:

- ✓ Removal of residual genomic DNA from RNA samples.
- ✓ Degradation of DNA template in transcription reactions.
- ✓ DNAse I footprinting.
- ✓ Perform Nick Translation.

Quality control:

- ✓ Functionally tested for digestion of template DNA after in vitro transcription.
- ✓ Confirmed absence of RNAse activity.
- ✓ Specific activity assayed by degradation of 1 µg of pUC18 in 40 mM Tris-HCl (pH 8.0), 10 mM $MgSO_4$, 1 mM $CaCl_2$.

Note:
One Kunitz unit equals to 50 Worthington units.

Proteinase K

Ordering info:

Cat No.	Size
EZ0011	30 mg
EZ0012	5 x 30 mg

Includes for 30 mg:

· 30 mg Proteinase K (Lyophilized powder)

Related products:

· HigherPurity™ Buccal Swab Genomic DNA Extraction Kit (p.92)
· HigherPurity™ Blood Genomic DNA Extraction Kit (p.94)
· HigherPurity™ FFPE DNA Isolation Kit (p.87)
· HigherPurity™ Bacterial Genomic DNA Isolation Kit (p.89)
· HigherPurity™ Stool DNA Isolation Kit (p.88)
· HigherPurity™ Blood Genomic DNA Extraction Mini Spin Kit (p.86)
· RNA services (p.140)

Description:

Proteinase K isolated from *Tritirachium album* is used for protease digestion during DNA and RNA preparation. It is a serine protease that exhibits broad cleavage specificity. With a molecular weight 28.900 kD, it cleaves peptide bonds adjacent to the carboxylic group of aliphatic and aromatic amino acids.

Proteinase K is not inactivated by chelating reagents such as EDTA or detergents such as SDS and is active over a wide range of pH (4-12.5).

Advantages & Features:

- ✓ **Highly active and stable:** > 30 units/mg protein (hemoglobin, pH 7.5, 37°C).
- ✓ **High Quality:** free of RNases, DNases and Exonucleases contamination.
- ✓ **Purified by chromatography.**
- ✓ **Lyophilised format.**
- ✓ **Low cutting specificity.**

Applications:

- ✓ Isolation of genomic DNA from cultured cells and tissues.
- ✓ Removal of DNases and RNases during DNA and/or RNA purification.
- ✓ Determination of enzyme locations.

Quality control:

- ✓ Enzyme activity is assayed by digesting hemoglobin at a concentration of 16.7 mg/mL in a solution of 0.08 M potassium phosphate (pH 7.5), 5M urea, 4 mM NaCl, 3 mM $CaCl_2$.
- ✓ Free of detectable RNase, DNase and exonuclease activities.

Exonuclease I *(E. coli)*

Ordering info:

Cat No.	Size
EZ0016	5,000 U
EZ0017	20,000 U

Includes for 5,000 U:

- 5,000 U Exonuclease I (20 U/µL)
- 375 µL Exonuclease I Reaction Buffer (10x)

Description:

Exonuclease I, the product of the sbcB gene of *E. coli*, is an exodeoxyribonuclease that hydrolyzes single-stranded DNA (ssDNA) stepwise in a 3´→5´ direction releasing 5'-mononucleotides and leaving the terminal 5'-dinucleotide intact.

Advantages & Features:

- **High Specific Activity:** 185,000 U /mg.
- **3' → 5' single strand exonuclease.**
- **Versatile:** suitable with a wide-range of Buffer conditions.
- **Compatible with magnesium.**
- **Complete solution:** supplied with 10x Exonuclease I Reaction Buffer.

Unit Definition:

One unit is defined as the amount of enzyme that will catalyze the release of 10 nmol of acid-soluble nucleotide in a total reaction volume of 50 µl in 30 minutes at 37°C in 1X Exonuclease I Reaction Buffer with 0.17 mg/ml single-stranded [3H]-DNA.

Applications:

- Removal of residual ssDNA, including oligos, from reaction mixes.

Quality control:

- Exonuclease I is tested in degradation of ssDNA and is free of detectable RNase, endonuclease and double stranded exonuclease activities.

Nucleotides

TruePure™ Nucleotides

Highest purity and stability for High-End PCR

Includes:
· TruePure™ dNTP

Advantages & Features:
- ✔ **Highest purity:** > 99% confirmed by HPLC.
- ✔ **Highly stable.**
- ✔ **Proven performance for PCR.**
- ✔ **High Quality:** free of DNAse, protease, phosphatase, nuclease, Human or *E. coli* DNA contamination.

Applications:
- ✔ For use in all common Molecular Biology applications, such as:
  - · PCR.
  - · Real-time PCR.
  - · High fidelity and long PCR.
  - · RT-PCR.
  - · cDNA synthesis.
  - · DNA sequencing.
  - · DNA amplification.

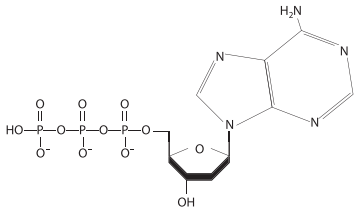
Quality control:
- ✔ TruePure™ dNTPs Certification: Functionally tested in 18 kb long range PCR (template dilution from 20 to 1,000 pg), with a proofreading enzyme and by RT-PCR.
- ✔ Exempt of DNAse, protease, phosphatase, nuclease, Human or *E. coli* DNA activities guaranteed by appropriate quality tests.

TruePure™ dATP

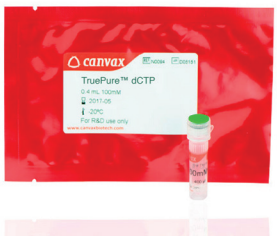
Ordering info:

Cat No.	Size
N0093-S	40 µmol: 0.2 mL of 100 mM solution
N0093	40 µmol: 0.4 mL of 100 mM solution

Specifications:
Formula: $C_{10}H_{16}N_5O_{12}P_3$
Molecular Weight: 491.18 g/mol
λmax pH 7.0= 259 nm
ε at λmax, pH 7.0= 15.4 mmol^{-1} cm^{-1}
Purity: > 99% confirmed by HPLC
Concentration: 100 mM
pH: 8.5



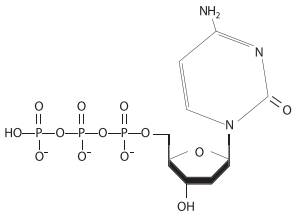
TruePure™ dCTP



Ordering info:

Cat No.	Size
N0094-S	40 µmol: 0.2 mL of 100 mM solution
N0094	40 µmol: 0.4 mL of 100 mM solution

Specifications:
Formula: $C_9H_{16}N_3O_{13}P_3$
Molecular Weight: 467.15 g/mol
λmax pH 7.0= 271 nm
ε at λmax, pH 7.0= 8.9 mmol^{-1} cm^{-1}
Purity: > 99% confirmed by HPLC
Concentration: 100 mM
pH: 8.5

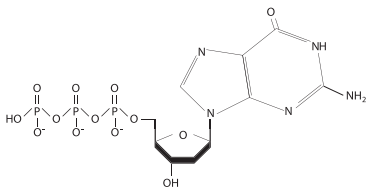


TruePure™ dGTP

Ordering info:

Cat No.	Size
N0095-S	40 µmol: 0.2 mL of 100 mM solution
N0095	40 µmol: 0.4 mL of 100 mM solution

Specifications:
Formula: $C_{10}H_{16}N_5O_{13}P_3$
Molecular Weight: 507.18 g/mol
λmax pH 7.0= 252 nm
ε at λmax, pH 7.0= 13.7 mmol^{-1} cm^{-1}
Purity: > 99% confirmed by HPLC
Concentration: 100 mM
pH: 8.5

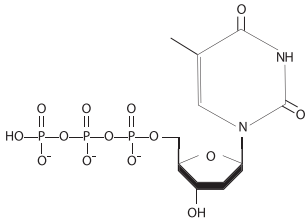


TruePure™ dTTP

Ordering info:

Cat No.	Size
N0096-S	40 µmol: 0.2 mL of 100 mM solution
N0096	40 µmol: 0.4 mL of 100 mM solution

Specifications:
Formula: $C_{10}H_{17}N_2O_{14}P_3$
Molecular Weight: 482.17 g/mol
λmax pH 7.0= 267 nm
ε at λmax, pH 7.0= 9.6 mmol^{-1} cm^{-1}
Purity: > 99% confirmed by HPLC
Concentration: 100 mM
pH: 8.5



Deoxynucleotides (dNTPs)

TruePure™ dNTP Mix

Description:

TruePure™ dNTP Mixes are solutions with each dNTP (dATP, dCTP, dGTP, dTTP) mixed at a final concentration of either 8, 10 or 100 mM total.

Advantages & Features:

- ✓ **Highest purity:** > 99% of each component confirmed by HPLC.
- ✓ **High Quality:** free of DNAse, protease, phosphatase, nuclease, human or *E. coli* DNA contamination.
- ✓ **Time-saving:** ready-to-use format that eliminates experiment preparation time.
- ✓ **Highly stable.**
- ✓ **Proven performance for PCR.**

Related products:

· Horse-Power™ Taq DNA Polymerase (p.103)
· FastPANGEA™ High Fidelity Polymerase (p.105)

Ordering info:

Cat No.	Size
N0030	5 x 1 mL 2 mM each (8mM total)
N0031	5 x 1 mL 2.5 mM each (10mM total)
N0032	1 mL 25 mM each (100mM total)

Includes:

· Premixed aqueous solutions of dATP, dTTP, dCTP and dGTP (pH 8.5)

TruePure™ dNTP set

Description:

TruePure™ dNTP set includes 4 vials, a separate vial of each dNTP (dATP, dCTP, dGTP, dTTP). Each dNTP are in aqueous solution at 100 mM.

Advantages & Features:

- ✓ **Highest purity:** > 99% of each component confirmed by HPLC.
- ✓ **Time-saving:** ready-to-use format that eliminates experiment preparation time.
- ✓ **Highly stable.**
- ✓ **Proven performance for PCR.**
- ✓ **High Quality:** free of DNAse, protease, phosphatase, nuclease, human or *E. coli* DNA contamination.

Related products:

· Horse-Power™ Taq DNA Polymerase (p.103)
· FastPANGEA™ High Fidelity Polymerase (p.105)
· AMV-RT (p.110)
· MMLV-RT (p.110)

Ordering info:

Cat No.	Size
N0098-S	4 x 0.2 mL of 100 mM
N0098	4 x 0.4 mL of 100 mM

Includes:

· 1 vial of each dNTP (dATP, dCTP, dGTP, dTTP) in aqueous solution at 100 mM each (pH 8.5)