

molecular biology

# ENZYMES

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**Thermolase™ # T1**

Modified *Thermus aquaticus* DNA polymerase.

**Description:** Thermolase™ (EC 2.7.7.7) is a thermostable DNA-dependent DNA polymerase cloned from the thermophilic eubacterium *Thermus aquaticus*. The enzyme has 5'-3' polymerase (60-150 nt/s, about 1 kb/min), 5'-3' exonuclease (strand displacement) and 3' terminal deoxynucleotidyl transferase activity (which usually results in addition of single dATP to the duplex DNA). Modified nucleotides (dNTPaS, c<sup>7</sup>GTP, biotin-11-dUTP, digoxigenin-11-dUTP and fluorescein-12-dUTP but not biotin-16-dUTP) are incorporated at high rates by the enzyme. Only the last three bases adjacent to the 3' end of the primer need to be correctly base paired, in order to initiate polymerization. The 5' region of the primer is less sensitive to base mismatches. Therefore, new restriction sites can be easily introduced into an amplification product. Up to 9 kb can be amplified from lambda DNA and up to 5 kb from genomic DNA.

**Unit definition:** One unit of the enzyme will incorporate 10 nmol of dNTPs into an acid-insoluble form in 30 minutes at 72°C under standard assay conditions using a DNA template. Specific activity is 200.000U/mg protein.

**Reaction buffer #B1** (x10, with Mg<sup>2+</sup>): 670 mM Tris-HCl, pH 8.8, 166 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 15 mM MgCl<sub>2</sub>, 0.1% Tween 20.

**Reaction conditions:** For sample volumes 20-100 µl use 200 µM dNTPs (end conc.), 25 pM (abs. quantity) each primer, 2 U of the enzyme, 100 ng genomic DNA as template. Addition of excess enzyme or template may lead to nonspecific amplification.

**Storage:** Store at -20°C.

**Applications:** PCR, mutation analysis (TaqMan™, PAMSA), site-directed mutagenesis, DNA labeling, 3' A-tailing of blunt ends; primer extension; sequencing.

**Quality control:** activity, SDS-PAGE purity, absence of endo- and exonucleases.

**References:** 1. Chien, A. et al. (1976) J. Bacteriol. 127, 1550. 2. Kaledin, A.S. et al. (1980) Biokhimiya 45, 494. 3. Clark, J.M. (1988) Nucleic Acids Res. 18, 9677-9686.

Size	Cat.#	Price EUR	Discount rate, %
1000 U	T1.4	100,00	0
2500 U	T1.5	237,50	5

**ThermolaseTA™ # T2**

Thermoactivated *Thermus aquaticus* DNA polymerase.

**Description:** ThermolaseTA™ (EC 2.7.7.7) is chemically modified *Thermus aquaticus* DNA polymerase that is inactive until incubation at 95°C for 15 min. During thermoactivation step the nonspecific primer annealing is prevented that results in increase of an amplification specificity.

**Unit definition:** One unit of the enzyme will incorporate 10 nmol of dNTPs into an acid-insoluble form in 30 minutes at 72°C.

**Reaction buffer #B35** (x10, with Mg<sup>2+</sup>): 670 mM Tris-HCl, pH 8.3, 160 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 15 mM MgCl<sub>2</sub>, 0.1% Tween 20.

**Storage:** Store at -20°C.

**Applications:** hot-start PCR.

**Quality control:** activity, SDS-PAGE purity, absence of endo- and exonucleases, specific performance test.

Size	Cat.#	Price EUR	Discount rate, %
1000 U	T2.4	200,00	0
2500 U	T2.5	475,00	5

**ThermolaseAB™ # T16**

Thermoactivated *Thermus sp.* DNA polymerase.

**Description:** ThermolaseAB™ (EC 2.7.7.7.) contains Thermolase™ DNA polymerase and antibody to provide automatic hot-start PCR. The antibody is used to block polymerase activity during set-up of the PCR reactions at ambient temperatures. The inhibition of DNA polymerase is completely reversed when the temperature is raised above 70°C. At the first template denaturation step in thermal cycling, the enzyme-antibody complex dissociates and the antibody is rendered nonfunctional. At the same time, the activity of DNA polymerase is restored, and the enzyme functions normally during the course of the PCR.

**Unit definition:** One unit of the enzyme will incorporate 10 nmol of dNTPs into an acid-insoluble form in 30 minutes at 72°C.

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**Reaction buffer #B1** (x10, with Mg<sup>2+</sup>): 670 mM Tris-HCl, pH 8.8, 166 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 15 mM MgCl<sub>2</sub>, 0.1% Tween 20.

**Storage:** Store at -20°C.

**Applications:** Hot-start protocol provides higher specificity and minimizes background. It is applied for amplification of complex cDNA templates, low-copy targets or in multiplex PCR.

**Quality control:** activity, SDS-PAGE purity, absence of endo- and exonucleases, specific performance test.

Size	Cat.#	Price EUR	Discount rate, %
1000 U	T16.4	200,00	0
2500 U	T16.5	475,00	5

### ThermolaseRed™ # T3

Modified *Thermus aquaticus* DNA polymerase with red dye in storage buffer.

**Description:** ThermolaseRed™ (EC 2.7.7.7) is formulated with cresol red dye in storage buffer for better visualization and loading of PCR products onto agarose gels. For further specifications, please refer to Thermolase™ #T1.

**Unit definition:** One unit of the enzyme will incorporate 10 nmol of dNTPs into an acid-insoluble form in 30 minutes at 72°C.

**Reaction buffer #B1** (x10, with Mg<sup>2+</sup>): 670 mM Tris-HCl, pH 8.8, 166 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 15 mM MgCl<sub>2</sub>, 0.1% Tween 20.

**Storage:** Store at -20°C.

**Applications:** PCR, mutation analysis, site-directed mutagenesis, DNA labeling, 3' A-tailing of blunt ends; primer extension; sequencing.

**Quality control:** activity, SDS-PAGE purity, absence of endo- and exonucleases, specific performance tests.

**References:** 1. Chien, A. et al. (1976) J. Bacteriol. 127, 1550. 2. Kaledin, A.S. et al. (1980) Biokhimiya 45, 494.

Size	Cat.#	Price EUR	Discount rate, %
1000 U	T3.4	100,00	0
2500 U	T3.5	237,50	5

### ThermolaseLR™ # T4

**Description:** ThermolaseLR™ (EC 2.7.7.7) enables consistent amplification of long DNA sequences. The mix combines DNA polymerases with different activities and properties to achieve faster and more accurate amplification of the DNA templates. The enzyme mix permits efficient amplification of up to 20 kb from lambda DNA and up to 15 kb from genomic DNA.

Although the size of the DNA is generally not a critical factor, amplification is improved if genomic DNA is digested with a rare cutting restriction enzyme (e.g., Not I). Target sequences are amplified slightly less efficiently when they are carried in closed circular form rather than in linear form. It is therefore preferable to linearize plasmid DNAs before they are used as templates in PCR.

**Unit definition:** One unit of the enzyme mix will incorporate 10 nmol of dNTPs into an acid-insoluble form in 30 minutes at 72°C.

**Reaction buffer #B4** (x10, with Mg<sup>2+</sup>).

**Storage:** Store at -20°C.

**Applications:** long-distance PCR.

**Quality control:** tested in long-distance PCR.

**References:** 1. Barnes, W.M. (1994) Proc. Natl. Acad. Sci. USA 91, 2216-2220. 2. Cheng, S. et al. (1994) Proc. Natl. Acad. Sci. USA 91, 5695-5699.

Size	Cat.#	Price EUR	Discount rate, %
500 U	T4.3	75,00	0
1000 U	T4.4	142,50	5

## ThermolasePR™ # T8

High-fidelity DNA polymerase with "proofreading" activity.

*Description:* ThermolasePR™ (EC 2.7.7.7) is a thermostable DNA-dependent DNA polymerase cloned from the archaea *Thermococcus litoralis* and overexpressed in recombinant *E.coli* strain. The enzyme has 5'-3' polymerase, 5'-3' exonuclease (strand displacement) as well as 3'-5' exonuclease ("proofreading") activities. The fidelity of ThermolasePR DNA polymerase is 10-fold greater as that of Taq DNA polymerase. The enzyme is extremely thermostable (half-life at 95°C 6.7 h, temperature optimum is 70-75°C).

*Unit definition:* One unit of the enzyme will incorporate 10 nmol of dNTPs into an acid-insoluble form in 30 minutes at 72°C under standard assay conditions using a DNA template.

*Reaction buffer #B1* (x10, with Mg<sup>2+</sup>): 670 mM Tris-HCl, pH 8.8, 166 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 15 mM MgCl<sub>2</sub>, 0.1% Tween 20.

*Storage:* Store at -20°C.

*Applications:* high-fidelity PCR; long-template and accurate (LA) PCR.

*Quality control:* activity, SDS-PAGE purity, specific performance tests.

*References:* 1. Mattila, P. et al. (1991) *Nucleic Acids Res.* 19, 4967-4973. 2. Kong, H.M. et al. (1993) *J. Biol. Chem.* 268, 1965-1975. 3. Perler, F. et al. (1992) *Proc. Natl. Acad. Sci. USA* 89, 5577. 4. Belkin, S., Jannasch, H.W. (1985) *Arch. Microbiol.* 141, 181-186.

## ThermolaseHF™ # T18

*Description:* ThermolaseHF™ (EC 2.7.7.7) enables amplification of the DNA with higher fidelity in comparison with Taq DNA polymerase. The mix combines DNA polymerases with different activities and properties to achieve more accurate amplification of the DNA templates. The enzyme mix permits efficient amplification of up to 10 kb from lambda DNA and up to 5 kb from genomic DNA.

*Unit definition:* One unit of the enzyme mix will incorporate 10 nmol of dNTPs into an acid-insoluble form in 30 minutes at 72°C.

*Reaction buffer #B21* (x10, with Mg<sup>2+</sup>).

*Storage:* Store at -20°C.

*Applications:* high-fidelity PCR.

*Quality control:* functionally tested in PCR.

*References:* 1. Lundberg, K.S. et al. (1991) *Gene*, 108, 1-6. 2. Cheng, S. et al. (1994) *Proc. Natl. Acad. Sci. USA* 91, 5695-5699.

Size	Cat.#	Price EUR	Discount rate, %
250 U	T8.2	125,00	0
500 U	T8.3	225,00	10

Size	Cat.#	Price EUR	Discount rate, %
500 U	T18.3	75,00	0
1000 U	T18.4	142,50	5

## ThermoKlen™ # T5

Modified *T. aquaticus* DNA polymerase lacking 5'-3' exonuclease activity.

**Description:** ThermoKlen™ (EC 2.7.7.7) is a N-terminally truncated *Thermus aquaticus* DNA polymerase where the domain responsible for the 5'-3' exonuclease activity is removed. This deletion does not distort the 3'-5' DNA-polymerase activity. As a result of this deletion, the enzyme is more heat-stable and is more suitable for amplification of GC-rich regions and templates with extensive secondary structures. The enzyme has a reduced tendency to extend a mismatched 3' oligonucleotide end that makes it more suitable for ARMS analysis. The enzyme has 3' terminal deoxynucleotidyl transferase activity which usually results in addition of single dATP to the duplex DNA. Optimal temperature is 70°C, half-life at 95°C is about 1 h.

**Unit definition:** One unit of the enzyme will incorporate 10 nmol of dNTPs into an acid-insoluble form in 30 minutes at 72°C.

**Reaction buffer #B5 (x10, with Mg<sup>2+</sup>):** 670 mM Tris-HCl, pH 9.1, 166 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 35mM MgCl<sub>2</sub>, 0.15% BSA.

**Storage:** Store at -20°C.

**Applications:** Real-time quantitative PCR with hybridization probes, mutation analysis, site-directed mutagenesis, ARMS analysis, DNA labeling, primer extension, sequencing.

**Quality control:** activity, SDS-PAGE purity, absence of endo- and exonucleases, specific performance tests.

**Reference:** 1. Barnes W.M. (1992) *Gene* 112, 29-35. 2. Wilhelm, J. et al. (2001) *BioTechniques* 30, 1052-1062.

## Tth DNA polymerase # T12

**Description:** Tth DNA polymerase (EC 2.7.7.7) is a thermostable DNA-dependent DNA polymerase derived from *Thermus thermophilus*. The enzyme is also capable of catalyzing the polymerization of DNA in the range of 1-2 kb using an RNA template in the presence of Mn<sup>2+</sup> ions. The ability of Tth DNA polymerase to reverse transcribe at elevated temperatures minimizes the problems encountered with strong secondary structures in RNA since they are unstable at higher reaction temperatures. Higher temperatures also result in increased specificity of primer hybridization and extension. Optimal temperature is 75°C, half-life at 95°C is 60 min. The enzyme will incorporate modified nucleotides (e.g., digoxigenin-11-dUTP and fluorescein-12-dUTP but not biotin-16-dUTP) at high rates. The enzyme has 3' terminal deoxynucleotidyl transferase activity which usually results in addition of single dATP to the duplex DNA.

**Unit definition:** One unit of the enzyme will incorporate 10 nmol of dNTPs into an acid-insoluble form in 30 minutes at 72°C.

**Reaction buffer.** Reverse transcription buffer #B6 (x10): 670 mM Tris-HCl, pH 8.8, 166 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.1% Tween 20. Supplied with 10 mM MnCl<sub>2</sub>. DMSO should be added at a final conc. of 5%. Amplification buffer#B9 (x5): 125 mM Tris-HCl pH 8.9, 12,5 mM MgCl<sub>2</sub>, 500 mM KCl, 3,75 mM EGTA, 0,05% gelatine, 0,25% Tween 20, 25% glycerol.

**Storage:** Store at -20°C.

**Applications:** RT-PCR.

**Quality control:** activity, SDS-PAGE purity, absence of endonucleases/nickases and exonucleases, specific performance tests.

**Reference:** 1. Rüttimann, C. et al. (1985) *Eur. J. Biochem.* 149, 41.

Size	Cat.#	Price EUR	Discount rate, %
1000 U	T5.4	100,00	0
2500 U	T5.5	237,50	5

Size	Cat.#	Price EUR	Discount rate, %
500 U	T12.3	100,00	0
1000 U	T12.4	180.00	10

## Tth inorganic pyrophosphatase # T11

*Description:* Tth inorganic pyrophosphatase (PPase, EC 3.6.1.1) catalyses the hydrolysis of inorganic pyrophosphate (which inhibits amplification reactions) to form orthophosphate:  $P_2O_7^{4-} + H_2O \rightarrow 2HPO_4^{2-}$ . The enzyme is extremely thermostable, retaining >90% activity after incubation at 100°C for 1 h.

*Unit Definition:* 1 unit will generate 40 nmol of phosphate per min from pyrophosphate under standard reaction conditions (50 mM Tricine pH 8.5, 1 mM MgCl<sub>2</sub>, 0,32 mM PPI at 75°C for 10 min).

*Reaction conditions:* The enzyme works well in all known polymerase buffers. Titrating the amount of the enzyme used in standard amplification reaction can result in significant improvements in DNA amplification. Recommended range of enzyme concentration is 1-100 mU per reaction.

*Storage:* Store at -20°C.

*Applications:* maintains forward direction of reactions generating pyrophosphate (e.g., high temperature sequencing, long-template PCR); oligo-labeling; removing CAP structure on mRNA, viral RNA and low molecular weight RNA, by cleaving the connecting 5'-5' triphosphate link; 5'- and 3'-end mapping of mRNA.

*Quality control:* activity, absence of ATPase and phosphatase activities, specific performance tests.

*References:* 1. Hohne, W.E. et al. (1988) Biomed Biochim Acta. 47, 941-947. 2. Satoh, T. et al. (1998) J. Biochem. (Tokyo) 124, 79-88.

## Tth DNA Ligase # T13

*Description:* Tth DNA ligase (EC 6.5.1.2) catalyzes the formation of a phosphodiester bond between juxtaposed 5' phosphate and 3' hydroxyl termini in duplex DNA structures that are stable at high temperatures. The enzyme is stable at elevated temperatures (half-life at 65°C is 48 h, and at 95°C 30 min). The enzyme will not join ss nucleic acids, blunt-ends and has no activity on dsRNA and RNA:DNA hybrids.

*Unit definition:* 1 unit ligates 50% of the cos sites in 1 µg λ DNA digested with Sma I per min at 45°C.

*Reaction buffer #B18 (x10):* 200 mM Tris-HCl pH 8.3, 250 mM KCl, 100 mM MgCl<sub>2</sub>, 5 mM NAD, 0,1% Triton X-100.

*Reaction conditions:* A typical 20 µl LCR reaction requires 5U of the enzyme. Optimum temperature is 24°-37°C for ligating cohesive termini and 65°-72°C for closing nicks.

*Storage:* Store at -20°C to +4°C at high concentration (>500U/ml).

*Applications:* mutation analysis (ligase chain reaction (LCR), repeat expansion detection (RED)); ligation under high-stringency hybridization conditions.

*Quality control:* activity, purity in SDS-PAGE, no detectable 5' exonuclease activity, tested in LCR.

*References:* 1. Schalling, M. et al. (1993) Nat. Genetics 4, 135. 2. Nakazawa, H. et al. (1994) Proc. Natl. Acad. Sci. USA 91, 360.

Size	Cat.#	Price EUR	Discount rate, %	Size	Cat.#	Price EUR	Discount rate, %
100 U	T11.1	50,00	0	500 U	T13.3	100,00	0
250 U	T11.2	112,50	10	1000 U	T13.4	190,00	5



## Thermonuclease™ # T14

*Description:* Thermonuclease™ is a thermostable endonuclease that recognizes and cleaves the junctions between single- and double-stranded DNA regions in partially duplexed DNA molecules. The DNA structure consisted of partially double-stranded hairpin regions interspersed with single-stranded regions is formed as a result of denaturation and subsequent cooling of ds DNA to an intermediate temperature.

*Unit definition:* One unit will digest 3.7 pmol of 33nt oligonucleotide substrate, which folds to create a 12-bp stem with a 3-nt loop, in 1 min at 55°C.

*Reaction buffer #B23:* (x10) 100 mM Tris-HCl, 20 mM MnCl<sub>2</sub>, 1% Triton X-100 for 0.1 - 1.0 kbp DNA fragments. In case of longer fragments (0,5-2 kbp), MgCl<sub>2</sub> should be added to the reaction mixture at a final conc. of 10 mM. Reaction can be performed also in the buffers used for thermostable polymerases.

*Storage:* Store at -20°C.

*Applications:* Cleavage Fragment Length Polymorphism (CFLP) technology.

*Quality control:* activity, SDS-PAGE purity.

*References:* 1. Lyamichev, V. et al. (1993) Science 260, 778-783. 2. Brow, M.A. et al. (1996) J. Clin. Microbiol. 34, 3129-37.

## Tth RNase H # T15

*Description:* Tth RNase H (EC 3.1.26.4) specifically degrades only RNA in an DNA:RNA hybrids, without affecting DNA or unhybridized RNA. The enzyme will not degrade ss or ds nucleic acids. The optimal temperature is 65°C. However, the enzyme works well at elevated temperatures up to 95°C.

*Unit Definition:* 1 unit results in the acid-solubilization of 1 nmol of <sup>3</sup>H radiolabelled poly(dT):poly(A) in 20 min at 45°C under standard reaction conditions.

*Reaction buffer #B25 (10x):* 200 mM HEPES-KOH pH 7.8, 500 mM KCl, 100 mM MgCl<sub>2</sub>, 10 mM DTT.

*Storage:* Store at -20°C.

*Applications:* high stringency hybrid selection; isotherm probe amplification; transcription-based amplification methods; high stringency mapping of mRNA structures; amplifications where specific removal of the RNA in a DNA:RNA hybrid is required.

*Quality control:* activity, absence of contaminating exo- and endonuclease activities.

*References:* 1. Duck, P. et al. (1990) Biotechniques 9, 142. 2. Bekkaoui, F. et al. (1996) Biotechniques 20, 240. 3. Guatelli, J.C. et al. (1990) Proc. Natl. Acad. Sci. USA 87, 1874.

Size	Cat.#	Price EUR	Discount rate, %	Size	Cat.#	Price EUR	Discount rate, %
500 U	T14.3	125,00	0	500 U	T15.3	450,00	0
1000 U	T14.4	225,00	10	1000 U	T15.4	810,00	10

## T4 gp32 # E1

*Description:* T4 gp32 is a single-strand DNA binding protein with M.W. approx. 33,5 kDa which stabilizes ss regions of DNA and RNA and destabilizes helices. It is reported to increase the specificity of PCR reactions and to improve the yield of long PCR products. Furthermore it is described to prevent inhibition of PCR caused by the presence of blood, to improve DNA sequencing of templates with secondary structures and to drive large scale restriction enzyme digests to completion.

*Reaction conditions:* T4 gp32 protein should be added to the reaction mixture at a final conc. of 1 nM.

*Storage:* Store at -20°C.

*Applications:* PCR, DNA sequencing, in studies of ssDNA-protein interactions and to visualize ssDNA by electron microscopy.

*Quality control:* activity, SDS-PAGE purity, absence of endo- and exonucleases.

*References:* 1. Cohen, H.A. et al. (1983) J. Mol. Biol. 169, 235-248. 2. Tsugita, A., Hosada, J. (1978) J. Mol. Biol. 122, 255-258. 3. Schwarz, K. et al. (1990) Nucl. Acids Res. 18, 1079.

## Nucleotides

2'-Deoxyribonucleoside-5'-triphosphate solutions.  
Biotin-11-dUTP.

*Description:* High quality solutions of dATP, dGTP, dCTP and dTTP are available either as individual nucleotides or as a mixture of all 4 nucleotides for use in DNA synthesis. Solutions are available at 2 mM (x10 dNTP mix), 20 mM (100x dNTP mix) and 100 mM (x500). 2 mM and 100 mM solutions of dUTP are also available for synthesis of uracil-containing DNA.

*Storage:* dNTP solutions are made with sterile deionized water and adjusted to pH 7.0 with NaOH. Store at -20°C.

*Applications:* DNA amplification, cDNA synthesis, sequencing, blunt end formation.

*Quality control:* all dNTP solutions meet spectrophotometric criteria and are functionally tested in PCR.

*References:* 1. Hoard, D.E., Ott, D.G. (1965) J. Am. Chem. Soc. 87, 1785-1788. 2. Yoshikawa, M. et al. (1967) Tetrahedron Letters 5065.

dATP	C <sub>10</sub> H <sub>16</sub> N <sub>5</sub> O <sub>12</sub> P <sub>3</sub>	M.W. 491,2
dGTP	C <sub>10</sub> H <sub>16</sub> N <sub>5</sub> O <sub>13</sub> P <sub>3</sub>	M.W. 507,2
dCTP	C <sub>9</sub> H <sub>16</sub> N <sub>3</sub> O <sub>13</sub> P <sub>3</sub>	M.W. 467,1
dTTP	C <sub>10</sub> H <sub>17</sub> N <sub>2</sub> O <sub>14</sub> P <sub>3</sub>	M.W. 482,1
dUTP	C <sub>9</sub> H <sub>15</sub> N <sub>2</sub> O <sub>14</sub> P <sub>3</sub>	M.W. 468,1

Premixed dNTP solutions. Supplied in a volume of 250 ml

# N1.2 20 mM solution of each dATP, dGTP, dCTP and dTTP (5µmol of each) 25,00 EUR

# N2.2 2mM solution of each dATP, dGTP, dCTP and dTTP (0,5µmol of each) 2,50 EUR

#N9.1 dNTP set 100 mM solutions of dGTP, dCTP, dATP, dCTP (25µmol of each) 80,00 EUR

Individual dNTP solutions. Supplied in a volume of 250 ml

# N3.2	100 mM solution of dATP (25µmol)	25,00 EUR
# N4.2	100 mM solution of dGTP (25µmol)	25,00 EUR
# N5.2	100 mM solution of dCTP (25µmol)	25,00 EUR
# N6.2	100 mM solution of dTTP (25µmol)	25,00 EUR
# N7.2	100 mM solution of dUTP (25µmol)	25,00 EUR
# N8.2	100 mM solution of biotin-11dUTP (25µmol)	25,00 EUR

Size	Cat.#	Price EUR	Discount rate, %
100 µg	E1.1	100,00	0
250 µg	E1.2	225,00	10

## Klenow fragment # L1

*Description:* Large fragment of E.coli DNA polymerase I (Klenow fragment, EC 2.7.7.7) possesses 5'-3' polymerase activity (45 nt/s), and 3'-5' exonuclease (proofreading) activity.

*Unit definition:* One unit of the enzyme converts 10 nmol of dNTPs to an acid-insoluble form in 30 min at 37°C.

*Reaction buffer #B19 (10x):* 100 mM Tris-HCl pH7.5, 50 mM MgCl<sub>2</sub>, 75 mM DTT.

*Reaction conditions:* For fill-in reaction DNA should be present at conc. 50 mg/ml. Add 1 U Klenow, 1 mM dNTPs (end conc.), reaction buffer and incubate 30 min at 37°C. Excessive amount of the enzyme or prolonged incubation time may result in recessed ends due to the 3'-5' exonuclease ("proofreading") activity.

*Storage:* Store at -20°C.

*Applications:* DNA radiolabeling by random priming, by filling-in reaction or by replacing of 3' nucleotides; fill-in protruding 5' termini for blunt-end formation; synthesis of the second cDNA strand.

*Quality control:* activity, absence of endonucleases, SDS-PAGE.

*References:* 1. Klenow, H. et al. (1971) Eur. J. Biochem. 22, 371-381.

## T4 DNA polymerase # L2

*Description:* T4 DNA polymerase (EC 2.7.7.7) catalyzes the 5'-3' synthesis of DNA from a primed ssDNA template. The enzyme can not utilize nicked DNA as a substrate, since it lacks 5'-3' exonuclease activity. The enzyme has a highly active 3'-5' proofreading exonuclease activity, 200-fold higher than that of Klenow fragment.

*Unit definition:* One unit of the enzyme catalyzes the incorporation of 10 nmol of total nucleotide into an acid insoluble form in 30 min at 37°C.

*Reaction buffer:* 10x buffer M

*Reaction conditions:* The enzyme will function in most buffers for restriction endonucleases, with pH optimum 8.0-9.0. The reaction can be performed at 37°C or at room temperature, but incubation at 12°C maximizes the polymerase activity over that of the exonuclease.

*Storage:* Store at -20°C.

*Applications:* removal of 3' overhang for blunt-end formation; „gap-filling“ during site-directed mutagenesis; single strand deletion subcloning; DNA labeling using replacement synthesis.

*Quality control:* activity, SDS-PAGE purity, absence of endonuclease activities.

*Reference:* 1. Englund, P. (1971) J. Biol. Chem. 246, 3269-3276.

Size	Cat.#	Price EUR	Discount rate, %
500 U	L1.3	70,00	0
1000 U	L1.4	133,00	5

Size	Cat.#	Price EUR	Discount rate, %
500 U	L2.3	70,00	0
1000 U	L2.4	133,00	5

## Reverse transcriptase MMLV H- # L3

*Description:* Reverse transcriptase M-MLV RNaseH- (EC 2.7.7.49) is a modified Moloney murine leukemia virus reverse transcriptase lacking RNase H activity. The enzyme displays low processivity and elongates DNA chains slowly and tends to pausing and termination during polymerization; error rate is low (1 mismatched residue in  $3 \times 10^4$  incorporated). In contrast to most other DNA polymerases, RT can use either RNA or DNA to prime DNA synthesis from an RNA or DNA template. M-MLV RNaseH- is much less sensitive than AMV RT to inhibition by tRNA and rRNA, and well suited for copying unfractionated mRNA.

*Unit definition:* 1 unit of the enzyme incorporates 1 nmol of dTTP into acid-insoluble form in 10 min at 37°C using 250  $\mu$ M poly(A)<sub>400</sub> and 25  $\mu$ M oligo(dT)<sub>50</sub>. The specific activity is approx. 100.000U/mg protein.

Reaction buffer #B16 (x5): 250 mM Tris-HCl pH 8.3, 150 mM KCl, 40 mM MgCl<sub>2</sub>, . Supplied with 100 mM DTT.

Reaction conditions: High concentration of dNTPs (>0,5 mM) in a reaction mixture results in the most efficient synthesis of full-length cDNAs. Spermidine-HCl at 0.5 mM stimulates the activity of AMV and Rauscher murine leukemia virus RT and inhibits M-MLV H-. DTT at a final conc. of 5-10 mM is necessary.

Storage: Store at -20°C. Freezing the enzyme does not affect the unit activity, but will decrease the functional ability to copy long mRNAs.

Applications: cDNA synthesis; production of strand-specific radiolabeled probes; filling in and labeling of DNA fragments with 5' protruding ends; blunt end formation during the second-strand reaction.

Quality control: activity, SDS-PAGE purity, absence of endonucleases/nickases, RNase and DNase activities, specific performance tests.

References: 1. Tanese N., Goff, S.P. (1988) Proc. Natl. Acad. Sci. USA 85, 1977. 2. Roth, M. et al. (1985) J. Biol. Chem. 260, 9326.

Size	Cat.#	Price EUR	Discount rate, %
20.000 U	L3.8	200.00	0
50.000 U	L3.9	400,00	20

## Reverse transcriptase enhancer # E3

*Description:* Reverse Transcriptase Enhancer (RTE) is an additive that can greatly increase the yield and specificity of reverse transcription. RT-Enhancer destabilizes hairpin RNA structure and activates annealing of the primer to RNA. The addition of RT-Enhancer generates greater yields in case of limited amounts of RNA using standard conditions, as well as results in a substantial increase in RT specificity and size of resulted product.

*Reaction conditions:* use 1 unit of RT-Enhancer per 20  $\mu$ l reaction.

*Storage:* Store at -20°C.

*Applications:* First strand cDNA synthesis for cloning and hybridization, RNA sequencing, RT-PCR.

*Quality control:* activity, SDS-PAGE purity, absence of endo- and exonucleases.

Size	Cat.#	Price EUR	Discount rate, %
500 U	E3.3	125,00	0
1000 U	E3.4	225,00	10

## SP6 RNA Polymerase # L4

*Description:* SP6 RNA polymerase (EC 2.7.7.6) is a DNA-dependent RNA polymerase that recognizes and initiates synthesis of RNA on dsDNA templates that carry the appropriate SP6 promoter (5'ATTTAGGTGACACTATA<sup>+</sup>1GAAGNG3'). The enzyme is a single polypeptide and is isolated from an overproducing recombinant E. coli strain.

*Unit definition:* One unit of the enzyme catalyzes the incorporation of 1 nmol of <sup>3</sup>H ATP into acid-insoluble product in 60 min at 40°C.

*Reaction buffer:* #B13 (x5).

*Reaction conditions:* 10 U SP6 polymerase, 40 mM Tris-HCl pH 7.5, 6 mM MgCl<sub>2</sub>, 2 mM spermidine-HCl, 10 mM DTT, 1 mM rNTPs, 2 µg template DNA. Additionally, inorganic pyrophosphatase can be added to a final concentration of 4 U/ml to enhance the polymerization reaction. The enzyme will incorporate <sup>32</sup>P, <sup>35</sup>S and <sup>3</sup>H nucleoside triphosphates.

*Storage:* Store at -20°C.

*Applications:* production of antisense RNA; RNA labeling; mRNA generation for in vitro translation.

*Quality control:* activity, SDS-PAGE purity, absence of RNase and DNase activities, specific performance tests.

*References:* 1. Butler, E.T., Chamberlin, M.J. (1982) J. Biol. Chem. 257, 5772. 2. Melton, D.A. et al. (1984) Nucl. Acids Res. 12, 7035.

## T7 RNA Polymerase # L5

*Description:* T7 RNA polymerase (EC 2.7.7.6) is a DNA-dependent RNA polymerase with a high specificity for T7 promoter sequences (5'TAATACGACTCACTATA<sup>+</sup>1GGGAGA3'). The enzyme is a single polypeptide and is isolated from an overproducing recombinant E. coli strain. RNA produced using the T7 RNA polymerase is biologically active as mRNA and can be accurately spliced

*Unit definition:* One unit of the enzyme catalyzes the incorporation of 1 nmol of <sup>3</sup>H ATP into acid-insoluble product in 60 min at 37°C.

*Reaction buffer:* #B13 (x5).

*Reaction conditions:* 10 U T7 polymerase, 40 mM Tris-HCl pH 7.5, 6 mM MgCl<sub>2</sub>, 2 mM spermidine-HCl, 10 mM DTT, 1 mM rNTPs, 2 µg template DNA. Additionally, inorganic pyrophosphatase can be added to a final concentration of 4 U/ml to enhance the polymerization reaction. The enzyme will incorporate <sup>32</sup>P, <sup>35</sup>S and <sup>3</sup>H nucleoside triphosphates. Under optimal conditions, greater than 700 moles of T7 RNA transcript can be synthesized per mol of DNA template.

*Storage:* Store at -20°C.

*Applications:* production of antisense RNA; RNA labeling; mRNA generation for in vitro translation.

*Quality control:* activity, SDS-PAGE purity, absence of RNase and DNase activities, specific performance tests.

*References:* 1. Stahl, S., Zinn, K. (1981) J. Mol. Biol. 148, 485. 2. Davanloo, P. et al. (1984) Proc. Natl. Acad. Sci. USA 81, 2035.

Size	Cat.#	Price EUR	Discount rate, %
2000 U	L4.5	100,00	0

Size	Cat.#	Price EUR	Discount rate, %
2000 U	L5.5	15.00	0

**T4 PNK # M4**

*Description:* T4 Polynucleotide kinase (EC 2.7.1.78) catalyzes the transfer of a  $\gamma$ -phosphate group from a 5'-nucleoside triphosphate moiety to a free 5'-hydroxyl of a polynucleotide such as DNA or RNA molecule and a nucleoside-3-monophosphate or deoxynucleoside-3-monophosphate. Excess of a nucleotide diphosphate such as ADP will cause the reverse reaction to be favored. That is used in phosphate exchange reaction for labeling of the DNA or RNA ends.

*Unit definition:* One unit of the enzyme catalyzes the incorporation of 1 nmol of phosphate to the 5'-OH end of an oligonucleotide from ( $\gamma$ -<sup>32</sup>P)ATP in 30 min at 37°C. The enzyme has specific activity in the range of 30.000-40.000 U/mg.

*Reaction conditions:* The phosphate group donor for the kinase reaction may be any nucleoside triphosphates at concentration >1  $\mu$ M. A total of 1.7 mM of spermine can increase reaction rate by 30-fold. Per reaction use 10-20 U PNK, 1-50 pmol DNA or 10 pmol of oligonucleotide, and 20 pM ( $\gamma$ -<sup>32</sup>P)ATP (end concentration of ATP should be at least 1  $\mu$ M).

Tris kinase buffer is used for kinasing of ssDNA fragments, duplexes with protruding 5' termini or labeling of oligonucleotides; the imidazole buffer is used for exchange reaction and kinasing DNA duplexes with blunt or recessed 5' ends. In exchange reaction, ADP and ATP should be added at a final conc. of 0,1 mM and 1 nM, respectively.

Tris Kinase Buffer #B11 (x10): 700 mM TrisHCl 7.6, 100 mM MgCl<sub>2</sub>, 50 mM DTT, 1 mM spermidine, 1 mM EDTA

Imidazole buffer #B12 (x10): 500 mM Imidazole-HCl pH 6.4, 180 mM MgCl<sub>2</sub>, 50 mM DTT, 1 mM spermidine, 1 mM EDTA.

*Storage:* Store at -20°C.

*Applications:* phosphorylation of the 5' termini and end labeling of DNA, RNA or synthetic oligonucleotides, removal of 3'-phosphoryl groups.

*Quality control:* activity, SDS-PAGE purity, absence of endonucleases/nickases.

*References:* 1. Richardson, C.C. (1965) Proc. Natl. Acad. Sci. USA 54, 158-163. 2. van de Sande, J.H. et al. (1973) Biochemistry 12, 5050-5055. 3. Lillehaug, J.R. et al. (1976) Biochemistry 15, 1858-1865.

Size	Cat.#	Price EUR	Discount rate, %
1000 U	M4.4	50,00	0
2500 U	M4.5	112,50	10

**T4 RNA ligase # M3**

*Description:* T4 RNA ligase (EC 6.5.1.3) forms a phosphodiester bond between a phosphorylated 5' terminus and a 3' hydroxyl of the ssRNA or ssDNA molecules. The minimum size of the 5' moiety is a ribonucleoside 3'5'-bis phosphate, a 5'-monophosphate being ineffective; the minimum size of the 3' moiety is a trinucleoside bis-phosphate.

*Unit definition:* 1 Unit ligates 1 pmol of <sup>32</sup>P-labelled mononucleoside biphosphate to a larger TCA-precipitable RNA species in 30 min at 4°C. Available at specific activities of 1000-4000 U/mg protein corresponding to 1-4 U/ $\mu$ l.

*Reaction buffer #B15 (x10):* 500 mM Tris-HCl 7.8, 100 mM MgCl<sub>2</sub>, 50 mM DTT, 10 mM ATP. Additional reagents: 50% PEG8000; 10 mM (NH<sub>4</sub>)<sub>3</sub>CoCl<sub>2</sub>.

*Reaction conditions:* 50 mM TrisHCl 8.0, 10 mM MgCl<sub>2</sub>, 20 mM DTT, 1 mM ATP, 10% DMSO, 10-100 mg/ml BSA, 1-16 U RNA ligase, 0,5-20  $\mu$ g RNA (1-100  $\mu$ M substrate termini), incubate at 37°C for 30 min (or at 4°C overnight); terminate by boiling for 2 min.

*Storage:* Store at -20°C.

*Applications:* cDNA cloning; synthesizing oligomers; 3' end-labeling of an RNA moiety; circularization of RNA and DNA oligonucleotides; joining RNA and ssDNA/dsDNA molecules.

*Quality control:* SDS-PAGE, absence of RNases.

*References:* 1. England, T. et al. (1977) Proc. Natl. Acad. Sci. USA 74, 4839-4842. 2. Tessier, D.C. et al. (1986) Anal. Biochem. 158, 171-178. 3. Zhang, X.H., Chiang, V.L. (1996) Nucl. Acids Res. 24, 990-991. 4. Kinoshita, Y. et al. (1997) Nucl. Acids Res. 25, 3747-3748.

Size	Cat.#	Price EUR	Discount rate, %
1000 U	M3.4	40,00	0
2500 U	M3.5	90,00	10

Continued on page 26

## T4 DNA Ligase # M6

**Description:** T4 DNA ligase (EC 6.5.1.1) catalyzes the formation of a phosphodiester bond between juxtaposed 5' phosphate and 3' hydroxyl termini in duplex DNA or RNA. The enzyme will not join ss nucleic acids. Detected activities include the joining of hydrogen-bonded cohesive DNA termini (with either 5' or 3' projecting single-stranded regions), the joining of blunt-ended double-stranded DNA molecules, the resealing of single-stranded „nicks“ in DNA duplexes, RNA or DNA/RNA hybrids, and the interconversion of ATP and AMP by the exchange of pyrophosphate groups.

**Unit definition:** 1 Weiss unit catalyzes the exchange of 1 nmol of <sup>32</sup>P from inorganic pyrophosphate to ATP in 20 min at 37°C. 0,01 Weiss units ligate >95% of the Hind III fragments from 1 mg lambda DNA in 30 min at 16°C.

**Reaction conditions:** 50 mM TrisHCl pH 7.6, 10 mM MgCl<sub>2</sub>, 10 mM DTT, 0,5-1 mM ATP, 50µg/ml BSA (optional), 0,1-1 WeissU/µg DNA. The enzyme has an optimum temperature for ligating cohesive ends of 4°C, for sealing nicks of 37°C and for blunt-end ligation of 25°C (if DNA fragments >16 bp long; smaller molecules requiring lower temperatures). The maximum effect for blunt-end ligation is achieved by the addition of PEG8000 to 15%, MgCl<sub>2</sub> to 5 mM and ATP to 0,5 mM. Similar effect is observed by addition of BSA to 100 µg/ml, hexammine cobalt chloride to 1.0-1.5 mM and spermidine to 0,1-1 mM. Blunt-end ligations require 10 to 100 times more enzyme than cohesive-end ligations to achieve an equal efficiency.

**Storage:** Store at -20°C.

**Applications:** construction of recombinant DNA molecules; detection of nicked DNA, joining RNA single strands via bridging oligonucleotide adapters.

**Quality control:** activity, SDS-PAGE purity, absence of RNase and DNase activities, mock cloning assay.

**References:** 1. Weiss, B. et al. (1968) J. Biol. Chem. 243, 4543. 2. Modrich, P. et al. (1972) J. Biol. Chem. 247, 6370-6372. 3. Murray, N.E. et al. (1979) J. Mol. Biol. 132, 493-505.

## Ribonuclease A # M11&M12

**Description:** Ribonuclease A (RNase A, EC 3.1.27.5) from bovine pancreas is a pyrimidine-specific endoribonuclease that acts on ssRNA (at NaCl conc. >0.3 M). However, if the conc. of RNaseA exceeds 2.2 KunitzU/ml, NaCl conc. <0.1 M and RNA conc. >30 µg/ml, then RNA strand in RNA:DNA duplexes as well as dsRNA will be cleaved. Cleavage occurs between the 3'-phosphate group of a pyrimidine ribonucleotide and the 5'-hydroxyl of the adjacent nucleotide. The reaction generates a 2':3' cyclic phosphate which then is hydrolyzed to the corresponding 3'-nucleoside phosphates. RNase A, which works in the absence of cofactors and divalent cations, can be inhibited by placental RNase inhibitor or by vanadyl-ribonucleoside complexes. M.W. approx. 13700. Activity 70-90 Kunitz U/mg protein.

**Unit definition:** One unit produces acid soluble oligonucleotides equivalent to ΔA260 of 1.0 per 30 min at 37°C and pH 7.5.

**Reaction conditions:** RNase A is active under an extraordinarily wide range of reaction conditions, and it is extremely difficult to inactivate. Use enzyme in excess (1-5U per ml of overnight bacterial culture (1x10<sup>9</sup> cells) or mammalian cell suspension (5x10<sup>7</sup> cells)) to degrade cellular RNA to completion. The optimal working concentration for degradative use is 50-150U/ml.

**Storage:** Store at -20°C.

**Applications:** mapping single-base mutations in DNA or RNA (mismatch analysis); removing unhybridized regions of RNA from RNA:DNA or RNA:RNA hybrids; removal of cellular RNA from DNA preparations; in conjunction with RNaseT1 in RNase protection assay; in conjunction with RNase H for blunt-ending ds cDNA.

**Quality control:** activity, minimum 70% by SDS-PAGE.

**References:** 1. Kunitz, M. (1946) J. Biol. Chem. 164, 563. 2. Laemmli, U.K. (1970) Nature 227, 680.

Size	Cat.#	Price EUR	Discount rate, %
1000 U	M6.4	40,00	0
2500 U	M6.5	90.00	10

Size	Cat.#	Price EUR	Discount rate, %
RNase A, boiled, supplied in storage buffer without glycerol			
1000 U	M11.4	5,00	0
RNase A, DNase free, supplied in storage buffer with glycerol			
500U	M12.3	5,00	0

## Proteinase K # P3

**Description:** Proteinase K (EC 3.4.21.14) is isolated from fungus *Tritirachium album* is a highly active serine protease of the subtilisin type. The enzyme catalyzes hydrolysis of a wide variety of peptide bonds but exhibits a preference for peptide bonds C-terminal to aromatic and uncharged amino acids. M.W. approx. 28930 Da. Activity approx. 30 U/mg protein.

**Unit Definition:** One Anson unit liberates 1µmol of Folin-positive amino acids per min at pH 7.5 and 35°C using haemoglobin as substrate.

**Reaction conditions:** Proteinase K is stable over a wide pH range (4-12.5); however, the enzyme is normally used in pH range 7.5-9.0. The activity of Proteinase K is severalfold higher at 50°C than at 37°C. Activity can be stimulated by addition of denaturing agents (0.5% SDS, 1% Triton X-100 and 1-4 M urea). The enzyme is inactivated by Pefabloc SC and inhibited by diisopropyl phosphofluoridate (DFP) and phenylmethanesulfonyl fluoride (PMSF). When Ca<sup>2+</sup> is removed from the enzyme, some of the catalytic activity is lost because of long-range structural changes. Because EDTA is a component of the buffer solutions used for RNA/DNA isolation, digestion is usually carried out under non optimal conditions. However, residual activity in a presence of EDTA is sufficient to degrade most proteins.

Use enzyme in excess (30-45U) to degrade 100 mg of solid tissue, pellet from 20 ml blood or 5x10<sup>7</sup> mammalian cells (1 ml suspension). The working concentration of the enzyme should be 3-6U/ml. Incubation time can range from 30 min to 18 h depending on the nature of the experiment.

**Reaction buffer:** B29 (x10): 500 mM Tris-HCl pH 7.5, 50 mM CaCl<sub>2</sub>. B30 (x10, for isolation of DNA and RNA from blood, cells and tissues): 100 mM Tris-HCl pH 8.0, 50 mM EDTA, 5% SDS.

**Storage:** Store at -20°C.

**Applications:** preparation of high-molecular weight DNA for PFGE and intact cellular RNA; protein fingerprinting; removal of nucleases from preparations of DNA and RNA; to study membrane protein topology and protein translocation across membranes.

**Quality control:** activity, SDS-PAG purity, no contaminating nuclease activity.

**References:** 1. Ebeling, W. (1974) Eur. J. Biochem. 47, 91-97. 2. Bajorath, J. et al. (1988) Eur. J. Biochem. 176, 441-447.

Size	Cat.#	Price EUR	Discount rate, %
500 U	P3.3	20,00	0
2500 U	P3.5	90,00	10

## Ribonuclease T1 # M13

**Description:** Ribonuclease T1 (RNase T1, EC 3.1.27.3) is isolated from an overexpressing *E.coli* strain containing the cloned *Aspergillus oryzae* gene. The enzyme specifically attacks the guanine sites in ssRNA to yield 3'-GMP and oligonucleotides with a 3'-GMP group. Activity approx. 500kU/mg protein.

**Unit definition:** One unit produces acid soluble oligonucleotides from yeast RNA equivalent to a ΔA260 of 1.0 per 15 min at 37°C and pH 7.5.

**Reaction conditions:** At low salt concentrations (<100 mM NaCl), it cleaves ss and dsRNA as well as the RNA strand in RNA:DNA duplexes. RNase T1 is active under a wide range of reaction conditions, and it is difficult to inactivate. Removal of RNase T1 from a reaction solution generally requires treatment with proteinase K followed by multiple phenol extractions and ethanol precipitation.

**Storage:** Store at +4°C.

**Applications:** determining the level of RNA transcripts synthesized in vitro from DNA templates containing a "G-less cassette"; in conjunction with RNase A in RNase protection assay; RNA fingerprinting; removing unhybridized regions of RNA from DNA:RNA or RNA:RNA hybrids.

**Quality control:** activity, minimum 70% SDS-PAGE purity, no detectable DNase and protease activities.

**Reference:** 1. Uchida, T., Egami, F. (1971) In The Enzymes, vol. IV, 205-250.

Size	Cat.#	Price EUR	Discount rate, %
100 kU	M13.1	50,00	0

Continued on page 30.



## DNase I, RNase free # M15

*Description:* Deoxyribonuclease I (EC 3.1.21.1), isolated from bovine pancreas, catalyzes the hydrolysis of dsDNA to produce 3'-hydroxyl oligonucleotides; at high concentration of the enzyme, ssDNA will also be digested. In the presence of Mg<sup>2+</sup> DNase I produces nicks in dsDNA, while in the presence of Mn<sup>2+</sup> the enzyme produces ds breaks. M.W. approx. 37000. Specific activity approx. 3000 Kunitz U/mg protein.

*Unit Definition:* One unit will cause an increase in absorbance of calf thymus DNA solution at a rate of 0,001 A260 units/min/ml at 25°C and pH 5.0.

*Reaction conditions:* 50 mM Tris-HCl pH 7.5, 10 mM MnCl<sub>2</sub>. (or 10 mM MgCl<sub>2</sub> in nick translation protocols), 50 µg/ml BSA, 1 µg DNA, 0,1-2 U of the enzyme (DNA degradation) or 20 mU (nick translation) in a 100µl reaction volume. Incubate at 37°C for 60 min. Stop reaction by adding EDTA to 25 mM (end conc.).

*Storage:* Store at -20°C.

*Applications:* nick translation; DNase footprinting, preparation of DNA-free RNA (RT-PCR, in vitro transcription/translation systems), cloning random DNA fragments by catalyzing ds cleavage of DNA in the presence of Mn<sup>2+</sup>.

*Quality control:* activity, SDS-PAGE purity, no contaminating ribonuclease activity.

*References:* 1. Kunitz, M. (1950) J. gen. Physiol. 33, 363. 2. Moor, S. (1981) In The Enzymes, vol 14A, 281-298. 3. Campbel, V.W., Jackson, D.A. (1980) J. Biol. Chem. 255, 3726-3735.

Size	Cat.#	Price EUR	Discount rate, %
2.500 U	M15.5	35,00	0
10.000 U	M15.7	133.00	5

## β-Agarase I # M16

*Description:* β-Agarase I (EC 3.2.1.81) is isolated from an overexpressing *E.coli* strain containing the cloned *Pseudomonas atlantica* gene. The enzyme specifically digests the agarose polysaccharide core made up of repeating 1,3-linked β-D-galactopyranose and 1,4-linked 3,6-anhydro-α-L-galactopyranose into neoagararoligosaccharides. M.W. approx. 32700. Specific activity approx. 25 U/mg protein.

*Unit Definition:* One unit will digest 100 µl of molten LMP agarose in 30 min at 40°C to completion.

*Reaction conditions:* The enzyme works equally well in different buffers (0.5xTBE, 1xTAE, 1xTPE or 1xBis-Tris); other recommendable buffer formulations include 1 M NaAc pH 5.0 (x10) or 100 mM Bis-Tris-HCl pH 6.5, 10 mM EDTA (x10).

*Storage:* Store at -20°C.

*Applications:* used for purification of nucleic acids from low melting point (LMP) agaroses.

*Quality control:* activity, SDS-PAGE purity, no contaminating nuclease and phosphatase activity.

*References:* 1. Yaphe, W. (1957) Can. J. Microbiol. 3, 987-993. 2. Schedl, A. et al. (1993) Nature 362, 258. 3. Steck, T.R. (1994) BioTechniques 17, 676.

Size	Cat.#	Price EUR	Discount rate, %
100 U	M16.1	100.00	0
500 U	M16.3	425.00	15

## TdT # M16

*Description:* Terminal deoxyribonucleotidyl transferase (EC 2.7.7.31) catalyzes the repetitive addition of mononucleotides from deoxynucleoside triphosphates to the terminal 3'-OH of a DNA initiator. ssDNA is preferred as an initiator. However, blunt or recessed 3' termini are used, albeit less efficiently, in buffers of low ionic strength that contain  $\text{Co}^{2+}$ ,  $\text{Mg}^{2+}$ , or  $\text{Mn}^{2+}$ . Will accept ddNTPs, succinylfluorescein-ddNTPs, rNTPs, biotin-11-dUTP, cordycepin triphosphate and phosphorothioate deoxynucleotide (dNTPaS) for terminal addition. Single nucleotides can be added to the 3' termini of DNA if modified nucleotides (rNTPs, ddNTPs, cordycepin triphosphate) are used as substrate.

*Unit Definition:* 1 unit catalyzes the transfer of 1 nmol of dATP to poly(dT)<sub>50</sub> in 1 h at 37°C.

*Reaction conditions:*

*Reaction buffer:* #B33 (x5): 500 mM Na cacodylate pH 7.2, 1 mM 2-mercaptoethanol, 10 mM  $\text{CoCl}_2$ .

*Resuspension and storage:* Dissolve at 20 mg/ml in sterile 50 mM Tris-HCl pH 8.0, 1.5 mM CaAc. Store aliquots at -20°C.

*Applications:* cloning; labeling of the 3'-hydroxyl ends of single- and double-stranded DNA, introduction of mispaired primer ends for site-directed mutagenesis protocols; apoptosis TUNEL assay; as a marker in leukemia diagnosis.

*Quality control:* activity, SDS-PAGE purity, absence of endonuclease/nickase, RNase and DNase activities, specific performance tests.

*References:* 1. Bollum, F.J. (1974) in *The Enzymes* (Boyer, P.D., ed.) Academic Press, NY, 145-171. 2. Deng, G., Wu, R. (1983) *Methods Enzymol.* 100, 96-116.

## CIAP # M9

Calf intestine alkaline phosphatase.

*Description:* Alkaline phosphatase (EC 3.1.3.1) purified from calf intestine is a glycoprotein with M.W of 100-140 kDa which catalyzes the removal of 5'- or 3'-phosphate from RNA, DNA and ribo- and deoxyribonucleoside di- or triphosphates. M.W. approx. 14000. Activity 3000 U/mg protein.

*Unit Definition:* One unit hydrolyzes 1  $\mu\text{mol}$  of 4-nitrophenylphosphate to 4-nitrophenol in 1 min at 37°C.

*Reaction conditions:* For dephosphorylation of DNA use 1 U of the enzyme for 1-20 pM DNA termini. Incubate in 1x reaction buffer for 30-60 min at 37°C. Stop reaction by heating at 75°C for 10 min. The DNA treatment with CIAP can be directly performed simultaneously or after the cleavage by a restriction endonuclease. In this case,  $\text{ZnCl}_2$  should be added to the reaction mixture at a final conc. of 1mM. Ends concentration for linear DNA can be approx. calculated as follows: pM ends = pM DNA x (number of cuts x2 + 2); 1  $\mu\text{g}$  of a 3 kb linear DNA contains 1 pM of 5' termini. Dephosphorylation of DNA with flush and recessed termini and dephosphorylation of RNA are more efficient performed at higher temperatures (50-60°C).

*Reaction buffer:* B22 (x10): 100 mM Tris-HCl pH 8.3, 10 mM  $\text{MgCl}_2$ , 10 mM  $\text{ZnCl}_2$ .

*Storage:* Supplied in storage buffer without glycerol. Store at +4°C.

*Applications:* used as a reporter in detection systems for proteins and nucleic acids detection; dephosphorylation of 5' termini of nucleic acids in cloning procedures to prevent self-ligation; subsequent tagging with radiolabeled phosphate using T4 PNK.

*Quality control:* activity in self-ligation test, SDS-PAGE purity, absence of contaminating nucleases.

*References:* 1. Engstrom, L. (1961) *Biochim. Biophys. Acta* 52, 36-48. 2. Mössner et al. (1980) *Z. Physiol. Chem.* 361, 543-549.

Size	Cat.#	Price EUR	Discount rate, %
500 U	M16.3	75,00	0
1000 U	M16.4	135,00	10

Size	Cat.#	Price EUR	Discount rate, %
1000 U	M9.4	50,00	0

## E. coli inorganic pyrophosphatase # M2

*Description:* E.coli inorganic pyrophosphatase (PPase, EC 3.6.1.1) catalyses the hydrolysis of inorganic pyrophosphate (which inhibits amplification reactions) to form orthophosphate:  $P_2O_7^{4-} + H_2O \rightarrow 2HPO_4^{2-}$ .

*Unit definition:* 1 unit will generate 40 nmol of phosphate per min from pyrophosphate under standard reaction conditions.

*Reaction conditions:* use 100-200 mU per reaction.

*Storage:* Store at -20°C.

*Applications:* in reactions, where pyrophosphate is accumulated (e.g., RNA synthesis with T7 RNA polymerase and reverse transcriptase, sequencing).

*Quality control:* assayed for the absence of ATPase activity, specific performance tests, SDS-PAGE purity.

*References:* 1. Lahti, R. et al. (1988) J. Bacteriol. 170, 5901-5907. 2. Salminen, T. et al. (1995) Biochemistry 34, 782-791.

## Uracil DNA glycosylase # M7

*Description:* Recombinant E.coli uracil-DNA glycosylase (UDG, Deoxy-D-cyclobutadiprimidine polynucleotido-deoxyribohydrolase, EC 3.2.2.17) catalyzes the release of free uracil from uracil-containing DNA. UDG efficiently hydrolyzes uracil from single-stranded or double-stranded DNA, but not from oligomers (6 or fewer bases) and RNA. This reaction leaves the DNA sugar-phosphodiester backbone intact. The resulting DNA is not suitable for use as hybridization target or as template for DNA synthesis.

*Unit definition:* One unit catalyzes the degradation of 1 µg ss uracil-containing DNA in 60 min at 37°C.

*Reaction conditions:* Treatment of 0,1 µg of uracil-containing DNA with 1 U of UDG for 10 min at 37°C renders the DNA incapable of being copied by DNA polymerase. The enzyme can be 95% irreversibly inactivated by incubation at 95°C for 10 min. The enzyme works well in buffers used in PCR and RT protocols.

*Storage:* Store at -20°C.

*Applications:* eliminates carryover contaminations that can result in false positives in PCR reactions; site-directed mutagenesis; cloning of PCR products.

*Quality control:* activity, SDS-PAGE purity, absence of exonuclease and unspecific endonuclease activities.

*References:* 1. Lingahl, T. et al. (1977) J. Biol. Chem., 252, 3286-3294. 2. Longo, M. et al. (1990) Gene 93, 125. 3. Wang, Z. et al. (1991) Gene 99, 31-37. 4. Devchand, P.R. et al. (1993) Nucl. Acids Res. 21, 3437-3443.

Size	Cat.#	Price EUR	Discount rate, %
500 U	M2.3	125,00	0
1000 U	M2.4	225,00	10

Size	Cat.#	Price EUR	Discount rate, %
500 U	M7.3	100,00	0
1000 U	M7.4	180,00	10

## S1 Nuclease # M1

*Description:* S1 Nuclease (EC 3.1.30.1) degrades ss DNA and RNA endonucleolytically to yield 5'-phosphoryl terminated products. Ds nucleic acids (DNA, RNA and DNA:RNA hybrids) are resistant to degradation with exception of highly concentrated enzyme. The digestion rate for ssDNA is 75.000 times faster than that of dsDNA. The enzyme will also cleave ss loops within RNA:DNA hybrids.

*Unit Definition:* 1 unit produces 1µg of acid-soluble material per min at 37°C using 0.5 mg/ml heat denatured calf thymus DNA as substrate.

*Reaction conditions:* For 100 µl reaction use 10 U S1 nuclease, 2 µg DNA, 50 mM NaAc pH 4.6, 1 mM ZnAc, 250 mM NaCl, 50 µg/ml BSA. Incubate at 37°C for 30 min. Stop reaction by addition of 1 µl 0.5 M EDTA. The reaction conditions will vary with different applications of S1 nuclease.

*Storage:* Store at -20°C.

*Applications:* mapping the 5' and 3' ends of RNA transcripts; digesting the hairpin loops formed during the synthesis of cDNA by reverse transcriptase; blunt end formation in ds DNA.

*Quality control:* activity, no detectable ds nuclease activity.

*References:* 1. Vogt, V.M. (1973) Eur. J. Biochem. 33, 192. 2. Roberts, T.M. et al. (1979) Proc. Natl. Acad. Sci. USA 76, 760-764. 3. Berk, A.J., Sharp, P.A. (1978) Proc. Natl. Acad. Sci. USA 75, 19274-1278.

Size	Cat.#	Price EUR	Discount rate, %
10.000 U	M1.7	30,00	0

## Exonuclease III # M5

*Description:* Exonuclease III (EC 3.1.11.2) catalyzes the stepwise removal of mononucleotides from 3'-OH termini of dsDNA. The preferred substrates are blunt or recessed 3' termini, although the enzyme also acts at nicks in dsDNA to produce ss gaps. The enzyme is not active on ssDNA, and thus 3'-protruding termini (longer as 4 nt) are resistant to cleavage. This property used to produce unidirectional deletions from a linear molecule with one resistant (3'-overhang) and one susceptible (blunt or 5'-overhang) terminus. Unidirectional deletions can also be created, if one terminus contains a-phosphorothioated nucleotide.

*Unit definition:* 1 unit produces 1 nmol of acid soluble nucleotides from dsDNA in 30 min at 37°C.

Reaction buffer #B27 (10x): 660 mM Tris-HCl pH 8.0, 6,6 mM MgCl<sub>2</sub>.

*Reaction conditions:* Digestion rate depends on reaction temperature, sequence, ionic strength and enzyme-to-DNA ratios. Mononucleotides are released as follow: C>A,T>G; digestion rate varies from 80bp/min at 25°C to 420 bp/min at 37°C.

*Storage:* Store at -20°C.

*Applications:* unidirectional nested deletions, site-directed mutagenesis, preparation of strand-specific probes.

*Quality control:* activity, absence of contaminating endonucleases.

*References:* 1. Rogers, S.G., Weiss, B. (1980) Meth. Enzymology 65, 201. 2. Henikoff, S. (1984) Gene 28, 351-359. 3. Guo, L.H, Wu, R. (1982) Nucl. Acids Res. 10, 2065-2084. 4. Vandeyar, M.A. (1988) Gene 65, 129-133.

Size	Cat.#	Price EUR	Discount rate, %
20000 U	M5.8	75,00	0

## E.coli RNase H # M10

*Description:* E. coli RNase H (EC 3.1.26.4) specifically hydrolyzes the phosphodiester bonds of RNA hybridized to DNA to produce 3'-hydroxyl and 5'-phosphate terminated products. It will not degrade single-stranded nucleic acids, duplex DNA or double-stranded RNA.

*Unit definition:* One unit is defined as the amount of enzyme required to produce 1 nmol of acid-soluble ribonucleotides from 0,5 nM radiolabeled poly(A):poly(dT) in 20 min at 37°C.

*Storage:* Store at -20°C.

*Applications:* Elimination of RNA during second strand cDNA synthesis; detection of RNA:DNA regions in dsDNA of natural origin; oligodeoxyribonucleotide-directed cleavage of RNA; removal of poly(A) sequences from mRNA in the presence of oligo(dT).

*Quality control:* activity, SDS-PAGE purity, absence of dsDNases, nickases and unspecific RNase activity.

*References:* 1. Gubler, U., Hoffman, B.J. (1983) Gene 25, 263-269. 2. Davis, R. et al. (1988) Mol. Cell Biol. 8, 4745-4755. 3. Donis-Keller, H. (1979) Nucl. Acids Res. 7, 179. 4. Goodwin, E.C., Rottman, F.M. (1992) Nucl. Acids Res. 20, 916.

## RNase inhibitor # M8

*Description:* Ribonuclease inhibitor (EC 2.7.7.49) from human placenta inhibits different RNase A-type enzymes (A, B, C) by binding noncovalently in 1:1 ratio. The enzyme does not inhibit RNase I, T1, T2, H, U1, U2, CL3 or S1 nuclease.

*Unit definition:* One unit inhibits 5 ng of RNase A with efficiency of 50% using cyclic 2',3' CMP as a substrate.

*Reaction conditions:* Ribonuclease inhibitor should be added to give a final concentration of 1U/μl. Presence of 1-10 mM DTT is required for activity. The enzyme can be inactivated by heating (>55°C), high salt concentration (e.g., 7 M urea) or addition of SDS.

*Storage:* Store at -20°C.

*Applications:* used in RNA isolation/purification protocols, in RT-PCR and in in vitro transcription/ translation.

*Quality control:* activity, SDS-PAGE purity, assayed in RT-PCR.

*Reference:* 1. Blackburn, P. (1979) J. Biol. Chem. 254, 12484.

Size	Cat.#	Price EUR	Discount rate, %
500 U	M10.3	400,00	0
1000 U	M10.4	720,00	10

Size	Cat.#	Price EUR	Discount rate, %
1000 U	M8.4	25,00	0
5000 U	M8.6	118,75	5

## Restriction enzymes

*Description:* Restriction enzymes are endonucleases that recognize specific ds DNA sequences and cleave the DNA in both strands. Three classes are recognized: Type I (EC 3.1.21.3) cleave the DNA at apparently random sites far away from the recognition site. Type III enzymes (EC 3.1.21.5.) cleave the DNA outside of the recognition site. Most characterized enzymes belong to the Type II class (EC 3.1.21.4); together with the Type IIS class they comprise the commercially available restriction enzymes used for DNA analysis and manipulation cleavage within the recognition site. Type II enzymes (EC 3.1.21.4) recognize symmetric DNA sequences and cleave within the sequences, leaving a 3'-hydroxyl on one side of the cut and a 5'-phosphate on the other. Type IIS enzymes recognize asymmetric and uninterrupted sequences, 4-7 bp in length. They cleave at a defined distance, up to 20 bp, to one side of their recognition sequence.

*Unit definition:* 1U is defined as the activity that cleaves 1 µg of lambda DNA in a 50µl reaction in 1 h under optimum buffer conditions at optimum temperature (usually 37°C).

*Reaction buffers:* Each enzyme supplied with a 10x reaction buffer:

Reaction buffer A (10X): 660 mM K-acetate, 330 mM Tris-acetate pH 7.9, 100 mM Mg-acetate, 5 mM DTT, 1 mg/ml BSA.

Reaction buffer B (10X): 1 M NaCl, 100 mM Tris-HCl pH 8.0, 50 mM MgCl<sub>2</sub>, 10 mM 2-mercaptoethanol, 1 mg/ml BSA.

Reaction buffer M (10X): 500 mM NaCl, 100 mM Tris-HCl pH 7.5, 100 mM MgCl<sub>2</sub>, 10 mM DTE, 1 mg/ml BSA.

Reaction buffer L (10X): 100 mM Tris-HCl pH 7.5, 100 mM MgCl<sub>2</sub>, 10 mM DTE, 1 mg/ml BSA.

Reaction buffer H (10X): 1 M NaCl, 100 mM Tris-HCl pH 7.5, 100 mM MgCl<sub>2</sub>, 10 mM DTE, 1 mg/ml BSA.

*Reaction conditions:* The preparation of DNA to be cleaved should be free of contaminants such as phenol, chloroform, alcohol, EDTA, detergents, or excessive salts, all of which can interfere with restriction endonuclease activity. The activity and specificity of some REs are influenced by the methylation state of the DNA substrate. To avoid site-specific dam and/or dcm methylation of recognition sites in E. coli vectors, it is necessary to propagate the vectors in strains lacking one or both of the methyltransferase systems. It must be taken into account, however, that E. coli strains, deficient in dam or dcm methylation, have elevated rates of mutation.

## Restriction enzymes (continued)

„Star“ activity is defined as a relaxed specificity of restriction enzymes under suboptimal reaction conditions (e.g. high glycerol concentration, low ionic strength or high pH values in the reaction buffer) and is related to the naturally observed limited accuracy of these enzymes when used in high concentration over a prolonged time. To keep glycerol concentration at less than 5% in a reaction, the restriction endonuclease should not exceed 10% of the total reaction volume.

Depending on the specific purpose, the digestion reaction is terminated by inhibiting, destroying or extracting the restriction enzyme. This can be achieved by chelation of the essential cofactor Mg<sup>2+</sup>, thermal denaturation (usually, at 65° to 95° for 10 min), or by phenol/chloroform extraction.

*Storage:* Store at -20°C.

*Applications:* analysis of the methylation state of genes (e.g., Hpa II vs Msp I); RLGS/RLCS, RFLP analysis; genetic fingerprinting; cloning, restriction mapping, DNA labeling.

Quality Control: activity, purity, absence of nonspecific endonucleases/exonucleases/other specific restriction endonucleases/phosphatases, ligation-recut assay.

References: 1. <http://rebase.neb.com>

Abbreviations: R=A or G; Y=C or T; W=A or T; D=A,G or T; N=G,A,T or C; \* = pronounced "star" activity; a = blocked by dam methylation; c = blocked by dcm methylation; CG = blocked at sites m<sup>5</sup>CG or m<sup>5</sup>CNG;

(CG) = cleavage blocked only at sites with overlapping CG methylation; ? = methylation sensitivity is not proven; iso = isoschizomers (recognize and cleave identical sites); neo = neoschizomers (enzymes having different cleavage specificity).

Cat.#	Enzyme iso/neo	Sequence	opt.T° Buffer	Price per 1 kU	Cat.#	Enzyme iso/neo	Sequence	opt.T° Buffer	Price per 1 kU
1588	Aat II	GACGT/C	37 A	40,00	1367	Not I	GC/GGCCGC	37 H	100,00
2113	Vha464 I • iso Afl	C/TTAAG	37 M	25,00	2673	CciN I • iso Not ICG	GC/GGCCGC	37 A	75,00
61	Alu	AG/CT	37 A	75,00	1371	Nru I	TCG/CGA	37 B	50,00
84	Apa I	GGGCC/C	37 A	10,00	2156	Zsp2 I • iso Nsi I	ATGCA/T	37 L	50,00
2683	PspOM I • neo Apa I	G/GGCCCC	37 A	25,00	4619	BstPA I • iso PshA I	GACNN/NGTTC	65 A	25,00
2116	Vne I • iso ApaL I	G/TGCAC	37 H	25,00	1536	Pst I	CTGCA/G	37 H	5,00
301	Bpu14 I • iso Asu II	TT/CGAA	37 M	25,00	1542	Pvu II	CAG/CTG	37 M	10,00
286	Bme18 I • iso Ava II	G/GWCC	37 H	25,00	1567	Rsa I	CT/AC	37 AL	50,00
185	BamH I	G/GATCC	37 ABM	2,50	2679	Psp124B I • iso Sac I	GAGCT/C	37 M	25,00
1182	Ksp22 I • iso Bcl I	T/GATCA	37 M	25,00	1662	Sfr303 I • iso Sac II	CCGC/GG	37 L	25,00
260	Bgl I	GCCNNNN/NGGC	37 H	25,00	1588	Sal I	G/TCGAC	37 H	10,00
261	Bgl II	A/GATCT	37 ABMH	25,00	2665	Acc113 I • iso Sca I	AGT/ACT	37 A	25,00
2681	PspE I • iso BstE II	G/GTNACC	37 L	25,00	1655	Sfi I	GGCCNNNN/NGGCC	50 M	25,00
2745	Bst2U I • iso BstN I	CC/WGG	60 M	25,00	1704	Sma I	CCC/GGG	25 A	25,00
716	Cla I	AT/CGAT	37 ABMH	50,00	2138	Xma I • neo Sma I	C/CCGGG	37 A	250,00
2671	Bsa29 I • iso Cla I	AT/CGAT	37 M	25,00	2667	AclN I • iso Spe I	A/CTAGT		150,00
778	Dra I	TTT/AAA	37 AM	50,00	1719	Sph I	GCATG/C	37 M	150,00
993	EcoR I	G/AATTC	37 ABH	2,50	2742	Sbf I • iso Sse8387	ICCTGCA/GG	37 A	150,00
995	EcoR V	GAT/ATC	37 B	10,00	1746	Ssp I	AAT/AAT	37 H	50,00
2744	BstF5 I • neo Fok I	GGATG(2/0)	65 A	50,00	1496	Pme55 I • iso Stu I	AGG/GCT	37 L	25,00
1056	Fok I	GGATG(9/13)	37 A	50,00	554	BssT1 I • iso Sty I	C/CWWGG	60 H	25,00
2743	BstH2 I • iso HaeII	RGCGC/Y	65 A	25,00	2674	Erh I • iso Sty I	C/CWWGG	37 H	25,00
620	BsuR I • neo Hae III	GG/CC	37 M	5,00	2750	Smi I • iso Swa I	ATTT/AAAT	37 H	100,00
1089	Hae III	GG/CC	37 M	10,00	1801	Taq I	T/CGA	65 AB	10,00
15	AccB1 I • iso HgiC I	G/GYRCC	37 extra	25,00	2639	Sse9 I • iso Tsp509 I	/AATT	55 L	150,00
2670	AspLE I • iso Hha I	GCG/C	37 H	25,00	2124	Vsp I	AT/TAAT	37 H	25,00
2675	HspA I • neo Hha I	G/CGC	37 A	25,00	2126	Xba I	T/CTAGA	37 H	10,00
1151	Hind III	A/AGCTT	37 BM	2,50	1661	Sfr274 I • iso Xho I	C/TCGAG	50 L	20,00
1154	Hinf I	G/ANTC	37 ABH	10,00	2136	Xho I	C/TCGAG	37 H	10,00
2862	BstHP I • iso Hpa I	GTT/AAC	37 A	50,00	2857	MroX I • iso Xmn I	GAANN/NTTTC	37 H	50,00
1159	Hpa II	C/CGG	37 L	25,00					
1277	Msp I • iso Hpa II	C/CGG	37 ABLM	10,00					
14	Acc65 I • neo Kpn I	G/GTACC	37 BH	50,00					
1180	Kpn I	GGTAC/C	37 L+BSA	10,00					
1186	Kzo9 I • iso Mbo Ia	/GATC	37 M	100,00					
1213	Mfe I	C/AATTG	37 L	100,00					
1236	Mlu I	A/CGCGT	37 H	25,00					
1817	Tru9 I • iso Mse I	T/TAA	65 ALM	100,00					
2676	MroN I • neo Nae I	G/CCGGC	37 L	25,00					
2858	Ege I • neo Nar ICG	GGC/GCC	37 L	100,00					
446	Bsp19 I • iso Nco I	C/CATGG	37 BH	25,00					
1308	Nco I	C/CATGG	37 H	75,00					
2736	FauND I • iso Nde I	CA/TATG	37 A	25,00					
2854	AsuNH I • iso Nhe I	TT/CGAA	37 L	100,00					

## Glutamylendopeptidase # P1

*Description:* Recombinant Glutamylendopeptidase (Endoproteinase Glu-C, EC 3.4.21.19) from *Bacillus intermedius* selectively hydrolyzes peptide bond formed by carboxyl moiety of glutamic acid. M.W. approx. 24500.

*Unit definition:* One unit hydrolyzes 1.0  $\mu$ mole of Z-Glu-pNA (carbobenzoxy-glutamyl-para-nitroanilide) per min at pH 7.2 at 37°C.

*Storage:* Store below 0°C.

*Applications:* used for selective cleavage of proteins for amino acid sequence determination or peptide mapping.

*References:* 1. Drapeau, G.R. (1977) Meth. Enzymol. 47, 189. 2. Cleveland, D.W. et al. (1977) J. Biol. Chem. 252, 1102. 3. Leshchinskaya, I.B. et al. (1997) FEBS Lett. 404, 241-244.

Two grades of purity are available:

P1.1. Single-step affinity chromatography

Electrophoretic purity 99%, contains 0.1% of non-specific activity of alkaline protease.

P1.2. Two-step affinity/FPLC chromatography

Electrophoretic purity - 100% (contaminations less than 0.01%, no concomitant protease activity).

## Carboxypeptidase B # P2

*Description:* Carboxypeptidase B (Protaminase; Peptidyl-L-lysine(L-arginine) hydrolase, EC 3.4.17.2) from porcine pancreas. M.W. approx. 34500.

*Unit definition:* One unit hydrolyzes 1.0  $\mu$ mole of hippuryl-L-arginine per min at pH 7.65 at 25°C. Activity approx. 175-250 units per mg protein.

*Storage:* Store below 0°C.

*Quality control:* Affinity purified. Lyophilized powder containing approx. 50-75% protein; balance primarily borat buffer salts, pH 7.5. Contains <1% carboxypeptidase A (hippuryl-L-phenylalanin), no detectable trypsin (BAPNA) and chymotrypsin (Z-Ala-Ala-Leu-pNA).

*Reference:* Akama, K., Tobita, T. (1987) Anal. Sci. 3, 167.

Two grades of purity are available:

P2.1. Lyophilized powder containing 50% protein.

P2.2. Lyophilized powder containing 75% protein.

Size	Cat.#	Price EUR	Discount rate, %
10 mg	P1.1	75,00	0
100 mg	P1.2	675,00	10
10 mg	P1.3	100,00	0
100 mg	P1.4	900,00	10

Size	Cat.#	Price EUR	Discount rate, %
10 mg	P2.1	100,00	0
100 mg	P2.2	900,00	10
10 mg	P2.3	125,00	0
100 mg	P2.4	1125,00	10



## Lambda DNA BstE II digest # S1

Size range 117- 14.140 bp.

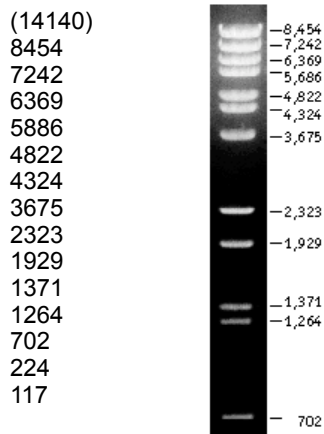
**Description:** The BstE II digest of lambda DNA (cl857 ind 1 Sam7) yields 14 fragments ranging in size from 117 bp to 8.454 bp. However, the cohesive ends of two fragments (8453 bp and 5687 bp) with cos sites may anneal to and form the additional band of 14.140 bp. Heating of the DNA to 75°C for 10 min followed by quick cooling will reversibly restore this band. When end labeled, lambda BstE II digest is suitable for evaluate the size of dsDNA in experiments based on chemiluminiscent and radioactive DNA detection.

**Storage:** Store at +4 to -20°C.

**Storage buffer:** 10 mM Tris-HCl pH 7.6, 1 mM EDTA.

**Application:** used in gel electrophoresis to evaluate the size of dsDNA fragments.

**Quality control:** spectrophotometric control, absence of contaminating ds nuclease activity, proper restriction fragments.



Size	Cat.#	Price EUR	Discount rate, %
Ready-to-use			
50 µg/100	S1.1	10,00	0
500 µg/1000	S1.2	90,00	10
Supplied with 6xloading dye			
50 µg/100	S1.3	10,00	0
500 µg/1000	S1.4	90,00	10

## 100 bp DNA Ladder # S2

Size range 100-1000 bp.

**Description:** The 100 bp DNA step ladder consists of 10 blunt-ended DNA fragments ranging from 100 to 1.000 bp in exact 100 bp increments. The marker can be optimally separated and analyzed in a 1% agarose gel stained with ethidium bromide. Load as much as 1 µg per lane (10 µl of ready-to-use marker). The marker can be internally labeled by nick translation protocol or end-labeled using TdT and T4 DNA polymerase or in phosphate exchange reaction using T4 PNK.

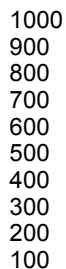
Supplied with 6xloading dye (40% Sucrose, 0,025% bromophenol blue, 0,025% xylene cyanol FF, 0,1% orange G). The xylene cyanol migrates with bands of approximately 4 kb, bromophenol blue with bands of approx. 300 bp and orange G with bands of approx. 50 bp in 0,5-1,4% agarose gels and 0,5xTBE.

**Storage:** Store at +4°C to -20°C. Avoid exposure to multiple freeze-thaw cycles.

**Storage buffer:** 10 mM Tris-HCl pH 7.8, 10 mM NaCl, 1 mM EDTA.

**Application:** used in gel electrophoresis to evaluate the size of dsDNA fragments.

**Quality control:** spectrophotometric control, absence of contaminating nucleases, accurate sizing.



Size	Cat.#	Price EUR	Discount rate, %
Ready-to-use			
50 µg/100	S2.1	30,00	0
500 µg/1000	S2.2	270,00	10
Supplied with 6xloading dye			
50 µg/100	S2.3	30,00	0
500 µg/1000	S2.4	270,00	10

## 1 kb DNA Ladder # S3

Size range 250-10.000 bp.

*Description:* The 1 kb DNA ladder consists of 13 blunt-ended DNA fragments ranging from 250 bp to 10 kb. The 1 kb and 3 kb bands are present at an increased intensity to simplify size assignment. The marker can be optimal separated and analyzed in a 1% agarose gel stained with ethidium bromide. Load as much as 1 µg per lane (10 µl of the ready-to-use marker). The marker can be internal labeled by nick translation protocol or end-labeled using TdT and T4 DNA polymerase or in phosphate exchange reaction using T4 PNK.

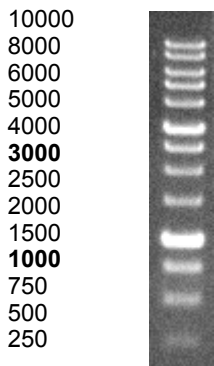
Supplied with 6xloading dye (40% Sucrose, 0,025% bromophenol blue, 0,025% xylene cyanol FF, 0,1% orange G)

*Storage:* Store at +4°C to -20°C. Avoid exposure to multiple freeze-thaw cycles.

*Storage buffer:* 10 mM Tris-HCl pH 7.8, 1 mM EDTA.

*Applications:* used in gel electrophoresis to evaluate the size of dsDNA fragments.

*Quality control:* spectrophotometric control.



Size	Cat.#	Price EUR	Discount rate, %
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Ready-to-use

50 µg/100

S3.1

30,00

0

500 µg/1000

S3.2

270,00

10

Supplied with 6xloading dye

50 µg/100

S3.3

10,00

0

500 µg/1000

S3.4

270,00

10

## Bacteriophage lambda DNA # D1

*Description:* Unmethylated cl857ind1Sam7 Bacteriophage lambda DNA (GenBank #NC\_001416) is isolated from infected GM 119, an E.coli strain lacking both dam and dcm methylase activities. The DNA is linear and double-stranded with 12 bp single-stranded complementary 5'-ends (cos sites). It has a molecular weight of 31,5x10<sup>6</sup> Da and contains 48.502 bp.

*Storage:* Store at +4°C. Upon storage at +4°C the DNA forms linear aggregates as well as circular structures due to the presence of cohesive termini. Heating of the DNA to 80°C for 10 min followed by quick cooling will reversible restore it to its linear form.

*Storage buffer:* 10 mM Tris-HCl pH 7.8, 10 mM NaCl, 1 mM EDTA.

*Applications:* used as substrate for restriction enzymes.

*Quality control:* spectrophotometric control, absence of contaminating ds nuclease activity, proper restriction fragments.

*References:* 1. Sanger, F. et al. (1982) J. Mol. Biol. 162, 729. 2. Arraj, J.A., Marinus, M.G. (1983) J.Bacteriol. 153, 562.

Size	Cat.#	Price EUR	Discount rate, %
500 µg	D1.3	50,00	0

## Phage T7 DNA # D2

*Description:* Phage T7 DNA (GenBank #V01146, 39.936 bp, M.W. approx.  $25 \times 10^6$ ) is isolated from E.coli B host strain ATCC11303. The DNA molecule is linear and double-stranded.

*Storage:* Store at +4°C to -20°C.

*Storage buffer:* 10 mM Tris-HCl pH 7.8, 1 mM EDTA.

*Applications:* as control template DNA in PCR; qualified as a marker for gel electrophoresis experiments and as substrate for restriction enzymes.

*Quality control:* spectrophotometric control, absence of contaminating ds nuclease activity, proper restriction fragments.

*References:* 1. Stahl, S.J., Zinn, K (1981) J. Mol. Biol. 148, 481-485. 2. Dunn, J.J., Studier, F.W. (1983) J. Mol. Biol. 166, 477-536.

## Human genomic DNA # D3

*Description:* High molecular weight genomic DNAs purified from blood or other nonglycogen-containing tissues to obtain intact genomic DNA with reduced turbidity. Based on modified salt precipitation procedure, DNA is recovered free of protein and RNA contaminations, on average is >100 kb in length, and has A260/A280 ratios 1.7-2.0. All donors of human genomic DNA was tested negative for HIV-1, HIV-2, HCV, HBV and syphilis (PCR, ELISA).

*Storage:* Store at +4°C.

*Applications:* Southern blot analysis, DNA amplification, genomic research.

*Quality control:* spectrophotometric control; absence of contaminating nucleases; tested for integrity in PFGE.

*References:* 1. Sambrook, J. et al. (1989) Molecular Cloning: A laboratory manual, CSH Laboratory, NY, 9.14. 2. Blin, N., Stafford, D.W. (1976) Nucl. Acids Res. 3, 2303-2308.

Size	Cat.#	Price EUR	Discount rate, %	Size	Cat.#	Price EUR	Discount rate, %
500 µg	D2.3	75,00	0	100 µg	D3.1 male	100,00	0
				100 µg	D3.2 female	100,00	0

## Human fetal RNA # R1

*Description:* Total RNA is purified from fetal tissues of human origine using modified guanidium thiocyanate method. During preparation, all tissues are rapidly minced and frozen in liquid nitrogen to prevent degradation by endogenous nucleases. All tissues used for RNA isolation are tested negative for HIV-1, HIV-2, HCV, HBV and syphilis (PCR or ELISA).

*Storage:* All preparations are provided in ethanol suspension containing 0.08 M sodium acetate. Store at -70°C.

*Applications:* cDNA synthesis; RT-PCR, Northern blot and primer extension analysis, RNase protection assay.

*Quality control:* spectrophotometric control, absence of contaminating ss and ds nuclease activity, high molecular weight DNA and proteins; each preparation contains intact 28S and 18S ribosomal RNA when electrophoresed on a denaturing agarose gel.

*References:* 1. Chirgwin, J.M. et al. (1979) *Biochemistry* 18, 5294-5299. 2. Chomczynski, P., Sacchi, N. (1987) *Anal. Biochem.* 162, 156-159.

## T-vector # K1

For cloning of dsDNA fragments with 3' single deoxyadenosine overhangs.

*Description:* The T-vector, based on the pBluescript KS+ cloning vector, is a ready-to-use linearized plasmid having single 3'-T overhangs on both ends. It is designed to ligate PCR products yielded with thermostable DNA polymerases possessing 3' terminal deoxynucleotidyl transferase activity that usually results in addition of a single deoxyadenosine to the 3'-end of PCR product. The vector can be used for cloning of PCR fragments generated by DNA polymerases with proof-reading activity, if these products are purified and finally incubated with appropriate DNA polymerase or with TdT and ddATP.

For subcloning purposes, sites for restriction endonucleases should be introduced into primers used for amplification by design. Cloned sequences can be transcribed using promoters for both T7 and T3 RNA polymerases flanking a cloning region.

*Storage:* Store at -20°C. Avoid exposure to multiple freeze-thaw cycles.

*Storage buffer:* 10 mM Tris-HCl pH 7.8, 1 mM EDTA.

*Applications:* cloning of DNA fragments with 3'-A overhangs.

*Quality control:* spectrophotometric control, cloning efficiency, self-ligation assay.

*References:* 1. Clark, J.M. (1988) *Nucleic Acids Res.* 18, 9677-9686. 2. Harrison J. et al. (1994) *Anal. Biochem.* 216, 235-236.

Size	Cat.#	Price EUR	Discount rate, %	Size	Cat.#	Price EUR	Discount rate, %
100 µg	R1	100,00	0	20 rxs	K1	75,00	0

## DNA purification kit # K2

For purification of PCR products and DNA fragments from agarose gels.

*Description:* DNA purification kit consists of 3 components: melting solution, DNA binding suspension and wash concentrate. DNA molecules >20 bp and up to 100 kb length can be purified with a recovery of >80%.

*Protocol:* 1. Add 23,75 ml deionized water and 25 ml ethanol to 1,25 ml wash concentrate to obtain 50 ml wash 1x solution. 2. Separate the DNA fragments by electrophoresis in 0,5-1xTAE agarose gel. Do not use TBE because borate is not compatible with melting solution. 3. Excise the DNA fragment from agarose gel and add 300-400 µl melting solution. Apply 600 µl for 2% agarose. Incubate at 50-60°C with shaking for 5 min to melt agarose. Cool down. For purification of PCR fragments, mix 20-100 µl PCR reaction with 300-500 µl melting solution. 4. Add 5-15 µl binding suspension (will bind 1-4 µg DNA), mix and centrifuge 1 min at 6.000 rpm. 5. Remove the supernatant. Add 500 µl of wash solution to the pellet and vortex. Repeat centrifugation. Wash twice. 6. Apply vacuum to dry the pellet. 7. Elute the DNA by resuspending the pellet in 50-100 µl deionized water. Incubate at 50-60°C for 5 min in shaker or vortex sometimes during incubation. Centrifuge 5 min at max. speed. 8. Transfer supernatant in a new tube. Repeat centrifugation step if necessary. Apply vacuum to concentrate the DNA.

*Storage:* Store melting solution in the dark at -20°C; DNA binding solution at +4°C and wash solution at room temperature. Vortex DNA binding solution vigorously every time before use.

*Applications:* purification of DNA fragments, PCR products and radiolabeled probes.

*Quality control:* spectrophotometric control of purified DNA, cloning efficiency after purification.

*Reference:* 1. Boyle, J.S., Lew, A.M. (1995) TIG 11, 8.

## Crystal violet solutions # K3

*Description:* DNA visualized by means of crystal violet solutions will not be damaged by ultraviolet (UV) light, as occurs when ethidium bromide is used for staining. The improvement in cloning efficiency is at least threefold as compared with ethidium bromide staining and UV wavelength approx. 320 nm and tenfold in comparison with shorter wavelength (302 nm).

*Protocol:* Crystal violet has a lower sensitivity than ethidium bromide. Load as much as 1 to 3 µg DNA on slot. Agarose gel should be prepared by addition of 100 µl Crystal Violet solution to 100 ml gel. Add 3-5 µl Crystal Violet loading buffer to 30 µl digest or PCR aliquot before loading on agarose gel. After electrophoresis, place gel on a light box for better visualization of the separated fragments. Crystal violet is running in opposite direction as the DNA. Don't run the gel too long time!

*Storage:* Store at +4°C to -20°C.

*Applications:* cloning of DNA fragments (PCR products and DNA molecules digested by restriction enzymes).

*Quality control:* spectrophotometric control purified DNA, cloning efficiency after purification.

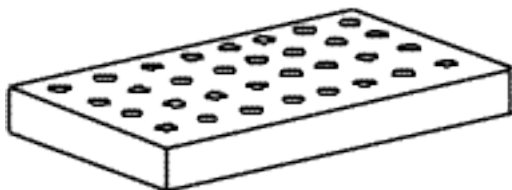
Size	Cat.#	Price EUR	Discount rate, %	Size	Cat.#	Price EUR	Discount rate, %
100 preps	K2	50,00	0	100 preps	K3	50,00	0

## CoolSafe™ aluminium racks #X1

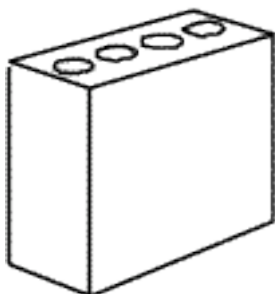
*Description:* Aluminium racks are designed to keep samples at low temperatures as well as heating, incubating and freezing. They are autoclavable and resistant to most chemicals. Each rack holds 32 x 1.5-2 ml tubes (X1.1), 4 x 15 ml tubes (X1.2) or 3 x 50 ml tubes (X1.3).

*Applications:* enzyme handling; preparation of competent cells, cell lysates and other temperature-sensitive probes.

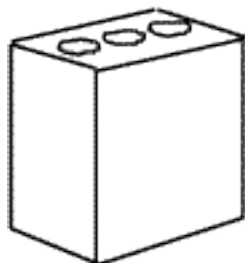
X1.1



X1.2



X1.3



Size	Cat.#	Price EUR	Discount rate, %
1 rack	X1.1	100,00	0
1 rack	X1.2	100,00	0
1 rack	X1.3	100,00	0

## Agarose Standard # Y1

*Description:* Agarose Standard is a high strength agarose for resolving DNA fragments between 500-20.000 bp. This agarose can be used in concentrations 0,8-2% with all common electrophoresis buffers.

*Specifications:* no detectable RNase/DNase activity; EEO 0,09-0,13; 1% gel strength >1200 g/cm<sup>2</sup>; 1.5% gel strength > 2500 g/cm<sup>2</sup>, gelling temp. 36±1,5°C; melting-point 88±1,5°C; moisture <7%; sulfate <0,20%.

*Storage:* Store at room temperature.

*Applications:* separation and further manipulation of DNA and RNA.

Size	Cat.#	Price EUR	Discount rate, %
250 g	Y1.2	150,00	0

## Agarose High standard # Y2

*Description:* Agarose High standard is an all purpose, low DNA binding agarose for routine nucleic acid electrophoresis of fragments between 100-50.000 bp. This agarose can be used in concentrations 0,4-2% with all common electrophoresis buffers.

*Specifications:* no detectable RNase/DNase activity; EEO <0,12; 1% gel strength >1800 g/cm<sup>2</sup> ; 1.5% gel strength >3200 g/cm<sup>2</sup>, gelling temp. 36±1,5°C; melting-point 88±1,5°C; moisture <7%; sulfate <0,12%.

*Storage:* Store at room temperature.

*Applications:* separation of proteins and nucleic acids (e.g., PFGE).

## Antibiotics # Y3-Y6

*Y3 Ampicillin* (D(-)-a-Aminobenzylpenicillin, C<sub>16</sub>H<sub>18</sub>N<sub>3</sub>NaO<sub>4</sub>S, M.W.= 371,39) is a half-synthetic penicillin (b-lactam antibiotic), known to interfere with the peptidoglycan cross-linking in grown bacteria and to inhibit cell wall biosynthesis. Acts at pH 5.5-6.0. Stock solution is prepared at conc. of 50 mg/ml in water; the working concentration is 20-50 µg/ml.

*Y4 Kanamycin A* (Kanamycin sulfate, C<sub>18</sub>H<sub>36</sub>N<sub>4</sub>O<sub>11</sub> • H<sub>2</sub>SO<sub>4</sub>, M.W.=582,58) binds to 70S ribosomal subunit, inhibits translocation and elicits miscoding. Stock solution is prepared at conc. of 10 mg/ml in water; the working concentration is 10-50 µg/ml. The solution is stable in pH range 6-8 at RT for 6-8 month (loss of activity approx. 1-2%); autoclaving at 120°C for 1 hour results in 5% loss of activity.

*Y5 Tetracycline* hydrochloride (C<sub>22</sub>H<sub>24</sub>N<sub>2</sub>O<sub>8</sub> • HCl, M.W.=480,90) inhibits protein synthesis (elongation) by preventing binding of aminoacyl-tRNA to ribosome. Do not use medium with magnesium for selection of bacteria resistant to Tc because Mg<sup>2+</sup> ions are antagonists of tetracycline. Stock solution is prepared at conc. of 10 mg/ml in 70% ethanol or in water; the working concentration is 10-50 µg/ml. Use freshly prepared stock solution; discard after 4 weeks of storage.

*Y6 G418* (Geneticin, C<sub>20</sub>H<sub>40</sub>N<sub>4</sub>O<sub>10</sub> • H<sub>2</sub>SO<sub>4</sub>, M.W.=692,70) is an aminoglycosidic antibiotic toxic to both pro- and eukaryotic cells. It acts by interfering with protein synthesis and is used for selection and maintenance of eucaryotic cells stably transfected with neomycin resistance gene. Stock solution is prepared at conc. of 40 mg/ml in PBS or water. The working solutions are 400 µg/ml for selection and 200 µg/ml for maintenance.

*Storage:* Store lyophilized powders at RT or at +4°C (G418), solutions at -20°C. Protect tetracycline from day light.

Size	Cat.#	Price EUR	Discount rate, %	Size	Cat.#	Price EUR	Discount rate, %
				5 g	Y3.1	7.50	0
				5 g	Y4.1	20,00	0
				5 g	Y5.1	2.50	0
250 g	Y2.2	250,00	0	1 g	Y6.1	120,00	0

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## Notes



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