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Other Enzymes for Molecular Biology

T4 DNA Ligase

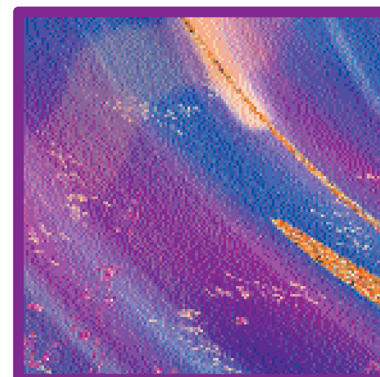
T4 RNA Ligase

T4 Polynucleotide Kinase

Exonuclease III

***Tli* Inorganic Pyrophosphatase**

DNA Polymerase I, *E.coli* (Klenow fragment)



T4 DNA Ligase

Description:

T4 DNA Ligase catalyzes the formation of a phosphodiester bonds between 5' phosphate and 3' hydroxyl termini in duplex DNA/RNA. This enzyme can join blunt end and cohesive end termini, repair single stranded nicks in duplex DNA, RNA or DNA/RNA hybrids.

Source:

Purified from *E. coli* strain harbouring the plasmid that directs the synthesis of T4 DNA Ligase.

Applications:

- Cloning of restriction fragments
- Joining linkers and adapters to blunt-ended DNA
- Gene (gene fragments) synthesis.

Cohesive End Ligation: For most cohesive end ligations, a 30 minute incubation at 20°C is sufficient. Incubations at 16°C for 4-16 hours are routinely used for the majority of applications.

Cat#	Pack size
402002	2000 U
402010	10000 U

Ligation of blunt ends and single-base pair overhang fragments requires more enzyme to achieve the same extent of ligation as cohesive end DNA fragments. Ligation may be enhanced by addition of PEG or by reducing the rATP concentration.
ATP is an essential cofactor for the reaction.

Concentration: 50000-100000 units/ml.

Storage Buffer:

50 mM KCl, 10 mM Tris-HCl (pH 7.4),
0.1 mM EDTA, 1 mM DTT, 50% glycerol.

Unit Definition:

One unit is defined as the amount of enzyme required to give 50% ligation of Hind III fragments of Lambda DNA in 30 minutes at 16°C with a 5' termini concentration of 0.12 µM (300 µg/ml). One Cohesive End Ligation Unit equals 0.015 Weiss units. One Weiss unit equals 67 Cohesive End Ligation Units. 2000 units of Bioron equals to 30 Weiss units.

Reaction Buffer (10x):

500 mM Tris-HCl (pH 7.8), 100 mM $MgCl_2$, 100 mM DTT, 10 mM ATP.

Storage Conditions: -20°C.

Quality Assurance:

Each lot of T4 DNA Ligase is tested for the absence of endonucleases and exonucleases.

T4 RNA Ligase

Description:

T4 RNA Ligase catalyzes the ATP-dependent ligation of single-stranded RNA or DNA onto the 5'-phosphoryl termini of single-stranded RNA or DNA. Substrates include single-stranded RNA and DNA as well as dinucleoside pyrophosphates. The enzyme has a molecular weight of 43.5 kDa.

Source:

Purified from *E. coli* strain containing the plasmid with T4 RNA ligase gene.

Applications:

- Labeling of 3' -termini of RNA with 5' -[^{32}P] pCp.
- Inter- and intramolecular joining of RNA and DNA molecules.
- Synthesis of single-stranded oligodeoxyribonucleotides.

Cat#	Pack size
404010	1000 U
404050	5000 U

Concentration: 50000-100000 units/ml.

Storage Buffer:

10 mM Tris-HCl (pH 7.4), 50mM KCl
0.1 mM EDTA, 1 mM DTT, 50% glycerol.

Unit Definition:

One unit is defined as the amount of enzyme required to convert 1 pmol of AMP to an acid-insoluble form in 10 minutes at 25°C.

Reaction Buffer (10X)

500 mM Tris-HCl (pH 7.8), 100 mM $MgCl_2$, 100 mM DTT, 10 mM ATP. Incubate at 37°C.

Storage Conditions: -20°C.

Heat Inactivation:

65°C for 15 minutes or boiling for 2 minutes.

BIORON

T4 Polynucleotide Kinase

Description:

T4 Polynucleotide Kinase (PNK) catalyzes the transfer of terminal phosphate from the gamma position of rATP to the 5' hydroxyl terminus of polynucleotides (double- and single-stranded DNA or RNA) and nucleoside 3' -monophosphates. PNK also catalyses the exchange of 5'-terminal phosphates and exhibits 3'-phosphatase activity.

Applications:

- End-labeling DNA or RNA
- Addition of 5' -phosphates to oligonucleotides
- Removal of 3' -phosphoryl groups.

Storage Buffer:

50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 0.1 µM ATP and 50% glycerol.

Unit Definition:

One unit is the amount of enzyme catalyzing the transfer of 1 nmol of phosphate in 30 minutes at 37°C.

Reaction Buffer (10x):

100 mM Tris-HCl (pH 7.5), 50 mM $MgCl_2$, 50 mM DTT.

The efficiency of blunt and recessed 5' -end phosphorylation can be improved by heating to 70°C for 5 minutes, then chilling on ice prior to kinase addition.

Since Polynucleotide Kinase is inhibited by ammonium ions, DNA should not be precipitated in the presence of ammonium ions prior to phosphorylation.

Quality Assurance:

Free of exonuclease, phosphatase, endonuclease and RNase activities.

Storage Conditions: -20°C.

Cat#	Pack size
403005	500 U
403025	2500 U

Exonuclease III

Description:

Exonuclease III is a 3'-5' exonuclease specific for double-stranded DNA. The enzyme catalyzes the stepwise removal of mononucleotides starting from a 3'-OH at nicks, blunt ends, recessed ends and 3'-overhangs of less than 4 bases, yielding nucleoside 5'-phosphates. A limited number of nucleotides are removed during each binding event, resulting in progressive deletions within the population of DNA molecules. Exonuclease III activity depends partially on helical structure and displays sequence dependence (C>A=T>G).

Temperature, salt concentration and the ratio of enzyme to DNA greatly affect enzyme activity, requiring reaction conditions to be adjusted to specific applications. Exonuclease III degrades DNA from 3'-phosphate ends due to its intrinsic 3'-phosphatase activity. In addition, the enzyme has apurinic endonuclease activity and ribonuclease H activity. Exonuclease III is used in conjunction with S1 nuclease for unidirectional deletion of sequences from the termini of DNA fragments.

Source:

Purified from *E. coli* strain harbouring the plasmid with *exoIII* gene.

Applications:

- Unidirectional nested deletions.
- Site-directed mutagenesis.
- Preparation of strand-specific probes.
- Preparation of single-stranded substrates for dideoxy sequencing.

Concentration: 40000-100000 units/ml.

Storage Buffer:

50 mM KCl, 10 mM Tris-HCl (pH 7.5), 0.5 mM EDTA, 1 mM 2-mercaptoethanol and 50% glycerol.

Unit Definition:

One unit is defined as the amount of enzyme required to produce 1 nmol of acid soluble nucleotides in 30 minutes at 37°C.

Reaction Buffer (10x):

500mM Tris-HCl (pH 7,6 at 30°C), 100 mM $MgCl_2$.

Storage Conditions: -20°C.

Heat Inactivation: 70°C for 20 minutes.

Cat#	Pack size
405040	4000 U
405200	20000 U

Tli Inorganic Pyrophosphatase

Description:

Inorganic Pyrophosphatase catalyzes the hydrolysis of inorganic pyrophosphate to form orthophosphate:
$P_2O_7 + H_2O \rightarrow 2HPO_4$

Source:

Purified from E.coli harbouring the pyrophosphatase gene of thermophile *Thermus litoralis*.

Cat#	Pack size
404305	20 U
404325	100 U

Applications:

●May be used in PCR to increase the yield of amplicons and in sequencing reaction to reduce the background.

Concentration: 1000 units/ml.

Unit Definition:

One unit is the amount of enzyme that generates 40 nmols of phosphate per minute from pyrophosphate at 65°C in 50 mM Tricine (pH 8.5) , 1 mM $MgCl_2$, 0.32 mM PPi.

Storage Conditions: -20°C.

DNA Polymerase I, Large (Klenow) Fragment

Description:

DNA Polymerase I, Large (Klenow) Fragment is a proteolytic product of *E. coli* DNA Polymerase I which retains polymerization and 3’→5’ exonuclease activity, but has lost 5’→3’ exonuclease activity. Klenow retains the polymerization fidelity of the holoenzyme without degrading 5’ termini.

Source:

E.coli strain harbouring the plasmid that directs the synthesis of Klenow fragment.

Applications:

●DNA sequencing by the Sanger dideoxy method.
●Fill-in of 5’ overhangs to form blunt ends.
●Removal of 3’ overhangs to form blunt ends.
●Second strand cDNA synthesis.
●Second strand synthesis in mutagenesis protocols.

Concentration: 5000 - 50000 units/ml.

Cat#	Pack size
401002	200 U
401010	1000 U

Storage Buffer:

100 mM KPO_4 (pH 6.5), 1 mM DTT and 50% glycerol.

Unit Definition:

One unit is defined as the amount of enzyme required to convert 10 nmols of dNTPs to an acid-insoluble form in 30 minutes at 37°C.

Recommended Reaction Buffer (10x):

100 mM Tris-HCl (pH 7.5), 50 mM $MgCl_2$, 50 mM DTT.

Klenow Fragment is also active in any restriction enzyme reaction buffer and T4 DNA Ligase reaction buffer when supplemented with dNTPs.

Quality Assurance:

Free of endonucleases and exonucleases .

Storage Conditions: -20°C.