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GenePharmaTM T4 DNA Ligase (Rapid)

Version 1.0

Catalog: J03002-1000
J03002-5000

Concentration:10 Weiss U/μl

Store at -20℃

Description:

T4 DNA Ligase Catalyzes the formation of a phosphodiester bond between juxtaposed 5'-phosphate and 3'-hydroxyl termini in duplex DNA or RNA. T4 DNA ligase catalyzes the repair of single-stranded nicks in duplex DNA, RNA or DNA/RNA hybrids and joins duplex DNA restriction fragments having either blunt or cohesive ends. The T4 DNA Ligase requires ATP as a cofactor.

Source: An Escherichia *coli* carrying plasmids that enable high expression of T4 DNA ligase.

Applications:

• Cloning of restriction enzyme generated DNA fragments.

•Cloning of PCR products.

•Nick repair in duplex DNA, RNA or DNA/RNA hybrids.

•Self-circularization of linear DNA..

•Joining of double-stranded oligonucleotide linkers and adapters to DNA.

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.6), 0.1 mM EDTA, 1 mM DTT and 50% glycerol.

Reagents Supplied with Enzyme:

2× Rapid Ligase Buffer.

10×T4 DNA Ligase Buffer

Reaction Conditions: 1×Rapid Ligase Reaction Buffer.

1×Rapid Ligase Reaction Buffer:

66 mM Tris-HCI, 10 mM $MgCl_2$, 1 mM DTT, 1 mM ATP, 7.5% PEG 6000, pH 7.6 @ 25℃.

1×T4 DNA Ligase Buffer:

50 mM Tris-HCI, 10 mM $MgCl_2$, 10mM DTT, 1 mM ATP, pH 7.6 @ 25℃.

Definition of Activity Unit

• One Weiss unit of the enzyme catalyzes the conversion of 1 nmol of [PPi] into Norit-adsorbable form in 20 min at 37℃.

• One Weiss unit is equivalent to approximately 200 cohesive end ligation units (CEU).

• One CEU is defined as amount of enzyme required to give 50% ligation of HindIII fragments of lambda DNA in 30 min at 16℃.

Heat Inactivation: Inactivated by heating at 65℃ for 10 min or at 70℃ for 5 min.

Quality Control Assays

Exonuclease Activity: Incubation of a 20 µl reaction in 1×Rapid Ligase Buffer containing a minimum 10 units T4 DNA Ligase with 500 ng human genomic DNA for 1 hour at 37°C without obvious degrade band by agarose gel electrophoresis.

Endonuclease Activity: Incubation of a 20 µl reaction in 1×Rapid Ligase Reaction Buffer containing a minimum 10 units T4 DNA Ligase with 1 µg of supercoiled PUC57 DNA for 1 hour at 37 ℃ results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

E.*coli* 16S rDNA Contamination Test： E.*coli* genomic DNA residue of 5 U T4 DNA Ligase after denatured is lower than 10 copy by E.*coli* 16s rRNA gene specific Real Time

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PCR detection with no template control.

Room Temperature Ligation:

For convenience, ligations may be done at room temperature (20 - 25°C). For cohesive (sticky) ends, use of T4 DNA Ligase in a 20 µl reaction for 10 minutes. For blunt ends, use 1 µl of T4 DNA Ligase in a 20 µl reaction for 2 hours or 1 µl high concentration T4 DNA Ligase for 10 minutes.

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.