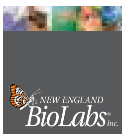
T3 DNA Ligase



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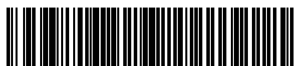


M0317S

100,000 units	3,000,000 U/ml	Lot: 0021303
RECOMBINANT	Store at –20°C	Exp: 3/15

Description: T3 DNA Ligase is an ATP-dependent ds DNA ligase from bacteriophage T3. It will catalyze the formation of a phosphodiester bond between adjacent 5´ phosphate and 3´ hydroxyl groups of duplex DNA. Cohesive ends, blunt ends, and nick sealing can all be efficiently catalyzed by T3 DNA Ligase (1). As with T4 DNA Ligase, blunt end ligation is enhanced by the addition of PEG 6000 to the reaction. T3 DNA Ligase exhibits a higher tolerance (2-fold) for NaCl in the reaction compared to T4 DNA Ligase, making the enzyme a versatile choice for *in vitro* molecular biology protocols requiring DNA ligase activity.

Source: An *E. coli* strain containing a recombinant gene encoding T3 DNA Ligase

Applications:
- Cloning of DNA fragments generated by restriction enzyme digestion
- Cloning of PCR products
- Adding linkers or adapters to dsDNA
- Circularization of linear DNA
- Nick-sealing in dsDNA
- Site-directed mutagenesis

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.4 @ 25°C), 0.1 mM EDTA, 1 mM DTT and 50% glycerol.

Reagents Supplied with Enzyme:
2X T3 DNA Ligase Reaction Buffer

Reaction Conditions: 1X T3 DNA Ligase Reaction Buffer. **Incubate at 25°C.** Standard vector + insert ligations in 10–20 µl reaction volumes are usually performed for 30 minutes at 25°C.

1X T3 DNA Ligase Reaction Buffer:
66 mM Tris-HCl
10 mM $MgCl_2$
1 mM ATP
1 mM DTT
7.5% PEG 6000
pH 7.6 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to give 50% ligation of 100 ng HindIII fragments of λ DNA in a total reaction volume of 20 µl in 1 minute at 25°C in 1X T3 DNA Ligase Reaction Buffer.

Concentration: 3,000,000 units/ml.

Heat Inactivation: No

Quality Control Assays

Exonuclease Activity: Incubation of 15,000 units of enzyme with 1 µg sonicated ^{3}H DNA (2 x 10^5 cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

Endonuclease Activity: Incubation of a 50 µl reaction containing 15,000 units of T3 DNA Ligase with 1 µg of ϕX174 RF I DNA for 4 hours at 37°C resulted in < 5% conversion to RFII as determined by agarose gel electrophoresis.

Physical Purity: Purified to > 99% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

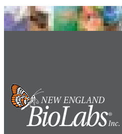
RNase Assay: Incubation of a 10 µl reaction containing 3,000 units of T3 DNA Ligase with 40 ng of 300 mer RNA transcript for 16 hours at 37°C resulted in < 10% degradation of RNA as determined by denaturing PAGE analysis.

Usage Notes: ATP is an essential cofactor for the reaction. This contrasts with *E. coli* DNA Ligase which requires NAD.

(see other side)

CERTIFICATE OF ANALYSIS

T3 DNA Ligase



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CERTIFICATE OF ANALYSIS

Dilution of enzyme for long-term storage at –20°C should be performed with the storage buffer containing 50% glycerol. Diluent A (NEB #B8001) can also be used for those application in which BSA, present in Diluent A, will not interfere.

T3 DNA Ligase is also active in buffers without PEG 6000, such as our T4 DNA Ligase Buffer and NEBuffers 1–4, for applications in which PEG 6000 is detrimental. Please remember to supplement the reaction with 1 mM ATP (final concentration). In these buffers T3 DNA Ligase exhibits an approximately 10-fold reduction in activity. In applications where a high concentration of NaCl needs to be maintained, we suggest using a reaction buffer without PEG 6000.

Heating a reaction containing T3 DNA Ligase at 65°C for 10 minutes will inactivate the enzyme. However, the reaction needs to be performed in a buffer without PEG. **Do not** heat inactivate if there is PEG in the reaction buffer, as transformation will be inhibited.

Reference:

1. Cai, L. et al. (2004) *J. Biochem.* 135, 397–403.

Companion Products Sold Separately:

Diluent A

#B8001S 10 ml

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