

T7 DNA Ligase



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M0318S 003160218021

M0318S



100,000 units **3,000,000 U/ml** **Lot: 0031602**
RECOMBINANT **Store at -20°C** **Exp: 2/18**

Description: T7 DNA Ligase is an ATP-dependent ds DNA ligase from bacteriophage T7. It will catalyze the formation of a phosphodiester bond between adjacent 5' phosphate and 3' hydroxyl groups of duplex DNA. Cohesive end ligation and nick sealing can be efficiently catalyzed by T7 DNA Ligase (1,2). However, unlike T4 and T3 DNA Ligases, blunt end ligation is not efficiently catalyzed by T7 DNA Ligase. Addition of high concentrations of PEG 6000 [$\geq 20\%$ (w/v)] to the reaction can force T7 DNA Ligase to have measurable activity. However, under typical

reaction conditions blunt-end DNA ligation does not occur in the presence of T7 DNA Ligase, making it a good choice for applications in which blunt and cohesive ends of DNA are present but only the cohesive ends are to be joined.

Source: An *E. coli* strain containing a recombinant gene encoding T7 DNA Ligase

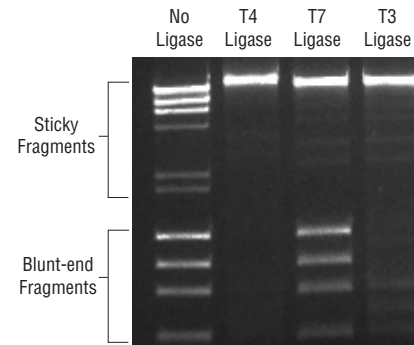
Applications:

- Cloning of DNA fragments generated by restriction enzyme digestion
- 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 T7 DNA Ligase Reaction Buffer

Reaction Conditions: 1X T7 DNA Ligase Reaction Buffer. **Incubate at 25°C.** Standard vector + insert ligations in 10–20 μ l reaction volumes are usually performed for 30 minutes at 25°C.



T7 DNA Ligase does not ligate blunt-end DNA fragments. Ligation reactions containing blunt fragments (ϕ X174 DNA-HaeIII Digest (NEB #N3026) and sticky-end fragments (λ DNA-HindIII Digest (NEB #N3012) were set up with 200 ng of each substrate and 1 μ l of each ligase, and incubated for 30 minutes at 25°C in their corresponding reaction buffers. Reactions were immediately stopped with 6X loading dye and resolved by electrophoresis on a 1% agarose gel and stained with ethidium bromide.

1X T7 DNA Ligase Reaction Buffer:

66 mM Tris-HCl
10 mM MgCl₂
1 mM ATP
1 mM DTT
7.5% PEG 6000
pH 7.6 @ 25°C

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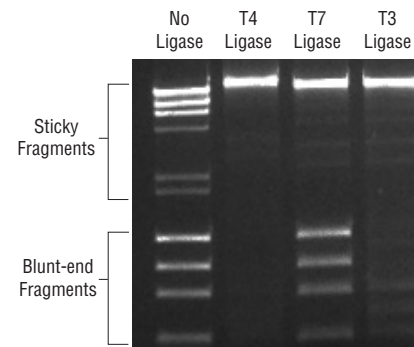
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1X T7 DNA Ligase Reaction Buffer:

66 mM Tris-HCl
10 mM MgCl₂
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 μ l in 30 minutes at 25°C in 1X T7 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 μ g sonicated ³H DNA (2 x 10⁶ cpm/ μ g) for 4 hours at 37°C in 50 μ l reaction buffer released < 0.1% radioactivity.

Endonuclease Activity: Incubation of a 50 μ l reaction containing 15,000 units of T7 DNA Ligase with 1 μ g of ϕ X174 RF I DNA for 4 hours at 37°C resulted in < 5% conversion to RFI as determined by agarose gel electrophoresis.

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

(see other side)

CERTIFICATE OF ANALYSIS

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Quality Control Assays

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Endonuclease Activity: Incubation of a 50 μ l reaction containing 15,000 units of T7 DNA Ligase with 1 μ g of ϕ X174 RF I DNA for 4 hours at 37°C resulted in < 5% conversion to RFI as determined by agarose gel electrophoresis.

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

(see other side)

CERTIFICATE OF ANALYSIS

RNase Assay: Incubation of a 10 µl reaction containing 3,000 units of T7 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.

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

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 applications in which BSA, present in Diluent A, will not interfere.

T7 DNA Ligase is also active in buffers without PEG 6000, such as our T4 DNA Ligase Buffer and NEBuffer 1-4, for applications in which PEG 6000 is detrimental. Please remember to supplement the reaction with 1 mM ATP (final concentration). Using these buffers, the activity of T7 DNA Ligase is reduced approximately 10-fold.

Heating a reaction containing T7 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.

References:

1. Doherty, A.J. et al. (1996) *J. Biol. Chem.* 271, 11083-11089.
2. Subramanya, H.S. et al. (1996) *Cell* 85, 607-615.



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