



T4 DNA Ligase

Cat. No. 15224-017

Size: 100 units

Cat. No. 15224-025

Size: 500 units

Conc.: 1 U/ μ l

Store at -20°C in a non- frost-free freezer.

Note: T4 DNA Ligase is unstable on ice for long periods. Therefore, Invitrogen recommends the enzyme be kept at -20°C until within 5-10 minutes of use and returned IMMEDIATELY to -20°C after use.

Description:

T4 DNA Ligase can be used to join DNA fragments with staggered or blunt ends and to repair nicks in double-stranded DNA having 3'-hydroxyl and 5'-phosphate ends. The enzyme is isolated from *E. coli* lambda lysogen NM989.

Unit Definition:

One (Weiss) unit catalyzes the exchange of 1 nmol 32 Pi into [γ/β - 32 P]ATP in 20 minutes at 37°C (1).

Components:

T4 DNA Ligase
5X DNA Ligase Reaction Buffer

Buffer Composition:

T4 DNA Ligase Storage Buffer:

10 mM Tris-HCl (pH 7.5)
50 mM KCl
1 mM DTT
50% (v/v) glycerol

5X DNA Ligase Reaction Buffer:

250 mM Tris-HCl (pH 7.6)
50 mM MgCl₂
5 mM ATP
5 mM DTT
25% (w/v) polyethylene glycol-8000.
Store buffer at -20°C.

Doc. Rev.: 052002

This product is distributed for laboratory research only. CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

Quality Control:

This product has passed the following quality control assays: functional absence of endonuclease and exonuclease activities: ligation/recut and ligation efficiency.

The enclosed buffers were assayed with the enzyme and met quality control specifications.

Protocols:

Note: Before use, thaw 5X DNA Ligase Reaction Buffer at room temperature and vortex vigorously to dissolve any precipitated material.

Recommended Conditions for General Cloning and Library Construction:

| | <u>Cohesive Ends</u> | <u>Blunt Ends</u> |
|----------------------------|----------------------|-------------------|
| 5X Ligase Reaction Buffer | 4 µl | 4 µl |
| Insert: Vector Molar Ratio | 3:1 | 3:1 |
| Vector Ends | 3-30 fmol | 15-60 fmol |
| Insert Ends | 9-90 fmol | 45-180 fmol |
| Total DNA | 0.01-0.1 µg | 0.1-1.0 µg |
| T4 DNA Ligase | 0.1 unit | 1.0 unit |
| Autoclaved distilled water | to 20 µl | to 20 µl |
| Temperature | 23-26°C | 14°C |
| Time | 1 h | 16-24 h |

Note: For optimal transformation, dilute the ligation reaction \geq 5-fold, to at least 100 µl, prior to adding to competent cells (2).

Rapid Ligation of Cohesive Ends (5-min) for Plasmid Cloning of DNA Fragments:

Note: The following protocol is for rapid ligation of cohesive ends. For rapid ligation of blunt ends, use T4 DNA Ligase, Cat no. 15224-041, which has a concentration of 5 U / μ l. This higher concentration is required for rapid ligation of blunt ends.

A molar ratio of 3:1 insert:vector is recommended for the rapid ligation of DNA inserts to vectors to produce circular recombinant molecules. Subsequent to restriction endonuclease digestion, purify the insert DNA from agarose using the S.N.A.P.TM Gel Purification Kit. Following restriction endonuclease digestion, dephosphorylate the vector DNA. Dephosphorylated vector can be used without purification if Calf Intestinal Alkaline Phosphatase (CIAP) is heat-inactivated prior to ligation.

1. To an autoclaved, 1.5-ml microcentrifuge tube, add the following:

| | <u>For Cohesive Ends</u> |
|----------------------------|--------------------------|
| 5X Ligase Reaction Buffer | 4 μ l |
| Vector DNA | 3 to 30 fmol |
| Insert DNA | 9 to 90 fmol |
| T4 DNA Ligase (units) | 1 unit (in 1 μ l) |
| Autoclaved distilled water | to 20 μ l |

2. Mix gently. Centrifuge briefly to bring the contents to the bottom of the tube.
3. Incubate at room temperature for 5 min.
4. Use 2 μ l of the ligation reaction to transform 100 μ l of MAX Efficiency[®] Competent cells.

References:

1. Weiss, B., Jacquemin-Sablon, A., Live, T.R., Fareed, G.C., and Richardson, C.C. (1968). *J. Biol. Chem.* 243, 4543.
2. Jesse, J. (1984). *Focus*® 6:4.