T4 DNA Ligase

Manual

Catalog #	3212	3216	3217
Package Size	100,000 units	100,000 units	400,000 units
Volume	250 µl	50 µl	200 µl
Concentration	400 units/µl	2,000 units/µl	2,000 units/µl

Important!

-20°C Storage Required

* Immediately inspect packages
* Freeze upon receipt

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Intact Genomics, Inc.

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Description:

Intact Genomics T4 DNA Ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5'-phosphate and 3'-hydroxyl termini in duplex DNA or RNA. This enzyme joins DNA fragments with either cohesive or blunt termini and repairs single stranded nicks in duplex DNA, RNA or DNA/RNA hybrids (1).

Protein Purity:

The physical purity of this enzyme is ≥99% as assessed by SDS-PAGE with Coomassie® blue staining (see figure below).

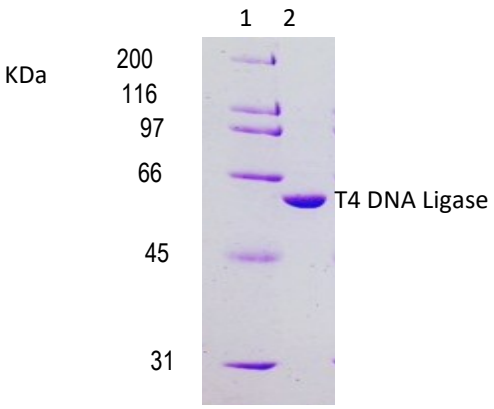

Fig: Lane 1. Protein marker
Lane 2. T4 DNA Ligase.

Product Source:

E. coli strain expressing a recombinant clone.

Product Components:

- T4 DNA Ligase
- 10x T4 DNA Ligase Reaction Buffer

Applications:

- Cloning of restriction enzyme generated DNA fragments
- Cloning of PCR products
- Next-gen library preparation
- Joining linkers and adapters to cohesive or blunt-ended DNA
- Nick repair in duplex DNA, RNA or DNA/RNA hybrids
- Self-circularization of linear DNA

Unit Definition:

One unit is defined as the amount of enzyme required to give 50% ligation of HindIII fragments of λ DNA (250 ng/µl) in a total reaction volume of 20 µl in 30 minutes at 16°C in 1X T4 DNA ligase reaction buffer.

1x T4 DNA Ligase Reaction Buffer:

50 mM Tris-HCl, 1 mM ATP, 10 mM $MgCl_2$, 10 mM DTT, pH 7.5 @ 25ºC

Storage Buffer:

50 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, pH 7.5 @ 25 ºC

Storage Temperature:

-20 ºC

Inhibition and Inactivation:

- Inhibitors: metal chelators, phosphate and ammonium ions, KCl and NaCl at a concentration higher than 50 mM.
- Inactivated by heating at 70 °C for 15 min or by addition of EDTA.

Ligation Protocol:

(400 units/µl T4 DNA Ligase concentration)

1) Set up reaction buffer in a microcentrifuge tube on ice. Use a molar ratio of 1:3 vector to insert DNA.

Component	20 µl reaction
Vector DNA	x µl
Insert DNA	x µl
10x T4 Ligase Buffer	2.0 µl
T4 DNA Ligase	1.0 µl
Add H_2O up to	20.0 µl

2) Gently mix the reaction and centrifuge briefly.
3) For cohesive ends, incubate at room temperature for 10 min or 16 ºC overnight.
4) For blunt ends, incubate at room temperature for 2 hours or 16 ºC overnight.
5) Heat inactivate at 70 ºC for 15 min.
6) Cool on ice and transform 2 µl of the reaction into 50 µl competent cells.

Related Products:

- T4 Polynucleotide Kinase (PNK) (Cat.# 3232)
- T4 DNA Polymerase (Cat.# 3222)
- ig® 10B Electrocompetent Cells (Cat.# 1212-12)
- ig® 10B Chemical Competent Cells (Cat.# 1012-12)
- ig® 5-alpha Electrocompetent Cells (Cat.# 1232-12)
- ig® 5-alpha Chemical competent Cells (Cat.# 1032-12)

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Our hours are Monday - Friday, 8AM to 5PM, U.S. Central Standard Time.

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