



LIFE TECH

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**IG Intact Genomics®**

## 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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### 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  $\geq 99\%$  as assessed by SDS-PAGE with Coomassie<sup>®</sup> blue staining (see figure below).

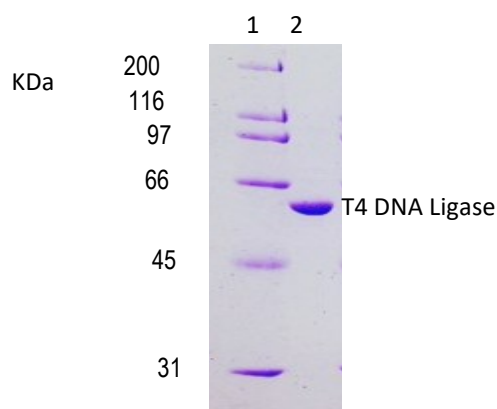


Fig: Lane 1. Protein marker  
Lane 2. T4 DNA Ligase.

### 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  $\lambda$  DNA (250 ng/ $\mu$ l) in a total reaction volume of 20  $\mu$ l 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<sub>2</sub>, 10 mM DTT, pH 7.5 @ 25°C

### Storage Buffer:

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

### Storage Temperature:

-20 °C

### 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/ $\mu$ l 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 $\mu$ l reaction
Vector DNA	x $\mu$ l
Insert DNA	x $\mu$ l
10x T4 Ligase Buffer	2.0 $\mu$ l
T4 DNA Ligase	1.0 $\mu$ l
Add H <sub>2</sub> O up to	20.0 $\mu$ l

- 2) Gently mix the reaction and centrifuge briefly.
- 3) For cohesive ends, incubate at room temperature for 10 min or 16 °C overnight.
- 4) For blunt ends, incubate at room temperature for 2 hours or 16 °C overnight.
- 5) Heat inactivate at 70 °C for 15 min.
- 6) Cool on ice and transform 2  $\mu$ l of the reaction into 50  $\mu$ l 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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