T4 DNA Ligase

Catalog #	3210	3212
Package Size	200 Weiss units	500 Weiss units
Volume	100 µl	250 µl
Concentration	2 Weiss units/µl	

Catalog #	3216	3217
Package Size	500 Weiss units	2,000 Weiss units
Volume	50 µl	200 µl
Concentration	10 Weiss units/µl	

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 enzymes joins DNA fragments with either cohesive or blunt termini as well as repair single stranded nicks in duplex DNA, RNA or DNA/RNA hybrids (1).

Physical Purity

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

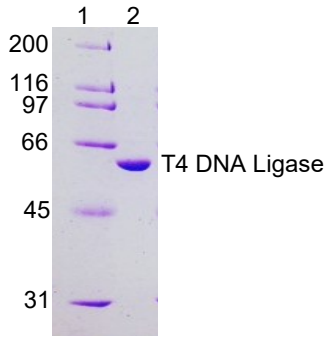

Figure: Lane 1. Protein Marker
Lane 2. T4 DNA Ligase

Product Source

E. coli strain expressing a recombinant clone

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

Product Includes

1) T4 DNA Ligase
2) 10x T4 DNA Ligase Reaction Buffer (w/o ATP)
3) 10 mM ATP

Storage Temperature

–20 °C

Storage Buffer

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

10x T4 DNA Ligase Reaction Buffer (w/o ATP)

500 mM Tris-HCl
100 mM $MgCl_2$
100 mM DTT
pH 7.5 @ 25 ºC

Note

10x T4 DNA ligase buffer does not contain ATP. You need to add ATP separately.

Unit Definition

One Weiss unit is defined as the amount of enzyme required to convert 1 nmol of ^{32}P from pyrophosphate into Norit-absorbance material in 20 minutes under standard assay conditions.

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

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

Component	10 µl Reaction
Vector DNA	x µl
Insert DNA	x µl
10 mM ATP	1.0 µl
10x T4 Ligase Buffer	1.0 µl
T4 DNA Ligase	1.0 µl
Add H_2O up to	10.0 µl

2) Gently mix the reaction and centrifuge briefly.
3) For cohesive ends, incubate 16 ºC for overnight or at room temperature for 30 min.
4) For blunt ends, incubate 16 ºC for overnight or at room temperature for 2 hrs.
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.

Quality Control Assays

- **Endonuclease Activity (Nicking)**

1 µg of supercoiled plasmid DNA is incubated with 20 units of T4 DNA Ligase in 1x Ligase buffer for 2 hours at 37 ºC. Following incubation, the supercoiled DNA is visualized on an ethidium bromide stained agarose gel. No visible nicking or cutting of DNA was found.

Intact Genomics®

- **Functional Assay**

DNA Ligase functional efficiency is tested in cloning assays.

Reference

1. Engler, M.J and Richadson, C.C (1982) In: The Enzymes, Boyer, P.D., ed., Academic Press, New York, NY.