

Intact Genomics

Higher Quality, Lower Price, Better Result

Enzymes & Cloning Kits

Cas9 Nuclease • Hot Start Taq DNA Polymerase • Hot Star Taq 2x Master Mix • Taq DNA Polymerase • Taq DNA Polymerase with Dye • Taq DNA Polymerase 2x Premix with Dye • Hot Start Pfu DNA Polymerase • Pfu 2x Master Mix • Pfu DNA Polymerase • igScript™ Reverse Transcriptase • ig™ SYBR Green qPCR 2x Master Mix • T4 DNA Ligase • Taq DNA Ligase • T4 DNA Polymerase • T4 Polynucleotide Kinase (PNK) • Klenow • Pol I • T5 Exonuclease • ig-Fusion™ Cloning Kit

RT-PCR & RT-qPCR Kits

igScript™ One Step RT-qPCR Kit • igScript™ First Strand cDNA Synthesis Kit • igScript™ RT-PCR Kit • igScript™ RT-qPCR Kit

Chemically & Electroporation Competent Cells

ig™ 5α • ig™ 10B • BL21 • BL21(DE3) • Phage Display Cells (TG1, SS320) • BAC Cells (10B, 10B Copy-Up) • Custom Cells

Custom Services

DNA Preparation: High HMW DNA • BAC DNA • High-Throughput DNA | **DNA Library Construction:** Random Shear BAC Library • Partial Digestion BAC Library • Fosmid Library | **Library Screening:** Colony Picking • Colony Duplication • 3D DNA Pools • Gridding & High Density Colony Filters • BAC/Fosmid end Sequencing | **Other Services:** Long DNA Fragment Cloning and Manipulation • BAC Engineering • Custom Vector Construction



T4 DNA Ligase

Catalog #	3210	3212
Package Size	20,000 units	100,000 units
Volume	50 µl	250 µl
Concentration	400 units/µl	

Catalog #	3216	3217
Package Size	100,000 units	400,000 units
Volume	50 µl	200 µl
Concentration	2000 units/µl	

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T4 DNA Ligase

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

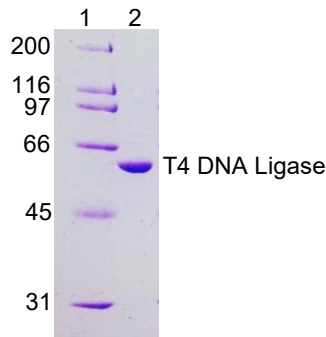


Figure: Lane 1. Protein Marker
Lane 2. T4 DNA Ligase

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

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 °C

1x T4 DNA Ligase Reaction Buffer

50 mM Tris-HCl
10 mM MgCl₂
10 mM DTT
1 mM ATP
pH 7.5 @ 25 °C

Unit Definition

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

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/ μ l T4 DNA Ligase conc.)

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

Component	20 μ l Reaction
Vector DNA	x μ l
Insert DNA	x μ l
10x T4 Ligase Buffer	2.0 μ l
T4 DNA Ligase	1.0 μ l
Add H ₂ O up to	20.0 μ l

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

Quality Control Assays

• Endonuclease Activity (Nicking)

1 μ g of supercoiled plasmid DNA is incubated with 2000 units of T4 DNA Ligase in 1x Ligase buffer for 2 hours at 37 °C. Following incubation, the supercoiled DNA is visualized on an ethidium bromide stained agarose gel. No visible nicking or cutting of DNA was found.

• Functional Assay

T4 DNA ligase functional efficiency is tested in cloning assay

Reference

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