TRIBI SCIENCE

T4 DNA Ligase (Catalog: TBS4025)

Catalog Number	Kit Size (KU)	Volume (µL)
TBS4025-20	20	50
TBS4025-100	100	250

Product Description

Tribiocience's 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 repairs single stranded nicks in duplex DNA, RNA or DNA/RNA hybrids.

Kit Content

Catalog Number	T4 DNA Ligase Concentration	Volume	10x Reaction Buffer
TBS4025-20	400 U/µL	50 µL	110 µL
TBS42020-100	400 U/µL	250 µL	550 µL

Storage and Shelf-life

Store at -25 ºC to -15 ºC immediately upon arrival. Minimize the number of freeze-thaw cycles to ensure superior performance. The Kit is stable for two (2) years from the date of arrival.

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.

Protein Purity

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

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 MgCl2, 10 mM DTT, pH 7.5 at 25ºC

Storage Buffer

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

Inhibition and Inactivation

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

Ligation Protocol

1. T4 DNA Ligation Preparation: Set up reaction mix buffer in a microcentrifuge tube on ice Using a molar ratio of 1:3 vector to insert DNA as Table 1.

Table 1: T4 DNA Ligation Reaction Preparation

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 for overnight.
4. For blunt ends, incubate at room temperature for 2 hours or 16 ºC for overnight.
5. Heat inactivate at 70º C for 15 min.
6. Cool on ice and transform 2 µl of the reaction into 50µl competent cells.

Related Products

T4 DNA Polymerase (TBS4026)
T4 Polynucleotide Kinase (PNK) (TBS4027)
One-Step RT-qPCR Kit (TBS4007)
Taqman qPCR Kit (TBS4002)
Sybro Green qPCR Kit (TBS4001)
Probe qPCR Kit (TBS4011)

Research Use Only

Tribioscience, Inc. 365 San Aleso Ave, Sunnyvale, CA 94085 www.tribioscience.com
Phone: 1-833-697-8998 (Toll Free) or 408-498-0197 (Office)