DATA SHEET

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T4 DNA Ligase

recombinant, *E. coli*

Cat. Nº.	Amount
■ ENZ-101S	100 Weiss units
□ ENZ-101M	250 Weiss units
□ ENZ-101L	2 x 250 Weiss units
□ ENZ-101XL	4 x 250 Weiss units

Unit Definition: One Weiss unit is defined as the amount of enzyme required to catalyze the exchange of 1 nmol of ^{32}P from pyrophosphate to ATP, into Norit-adsorbable material in 20 minutes at 37°C.

Storage Buffer
The enzyme is supplied in: Tris-HCl (pH 7.4), KCl, EDTA, glycerol and stabilizers.

Concentration:
2.5 WU/µL

For *in vitro* use only!

Shelf Life:
12 months

Shipping:
Shipped on blue ice

Storage Conditions:
Store at -20 °C

Additional Storage Conditions:
Avoid freeze/thaw cycles.

Notes:

• One Cohesive-End Ligation Unit (CEU) is defined as the amount of enzyme required to give 50% ligation of Hind III fragments of lambda DNA (5' DNA termini concentration of 0.12 µM, 300 µg/ml) in a total reaction volume of 20 µl in 30 minutes at 16 °C in 1x T4 DNA Ligase Reaction Buffer.

• One Weiss unit is equivalent to approx. 67 CEU.

• T4 DNA Ligase is strongly inhibited by NaCl or KCl if the concentration exceeds 200 mM.

• Ligation of blunt-ended and single-base pair overhang fragments requires about 50 times as much enzyme to achieve the same extent of ligation as cohesive-end DNA fragments. Blunt-end ligation may be enhanced by using 2X Fast Ligation Buffer.

• To dilute T4 DNA Ligase for subsequent storage at -20 °C a storage buffer containing 50 % glycerol should be used; to dilute Ligase for immediate use, 1x Reaction Buffer is recommended.

Description:
T4 DNA Ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5' phosphate and 3'-hydroxyl termini in duplex DNA or RNA.

Reaction conditions:
For a typical ligation of 100 - 200 ng vector DNA 1 - 5 Weiss Units of T4 DNA Ligase are used. Optimal ligation occurs at 16°C.

10 X Reaction Buffer (2 ml/250 WU)
Tris-HCl pH 7.8 at 25 °C, 100 mM $MgCl_2$, DTT, ATP and stabilizers.

2 X Fast Ligation Buffer (2 ml/250 WU)
Tris-HCl pH 7.8 at 25 °C, 20 mM $MgCl_2$, DTT, ATP and stabilizers.
(Ligation occurs within 5 minutes for cohesive-ended ligations or 15 minutes for blunt-ended ligations)

Protocol for Ligation of DNA
We recommend using a 1:1, 1:3 or 3:1 molar ratio of vector:insert DNA when cloning a fragment into a plasmid vector. Typical ligation reactions use 100–200ng of vector DNA.

1. Standard Ligation
Assemble the following reaction in a sterile microcentrifuge tube. Example for a 1:1 vector:insert ratio:

Component	20 µL reaction
Ligase 10X Buffer	2 µL
Vector DNA (3 kb)	100 ng
Insert DNA (0.5 kb)	17 ng
T4 DNA Ligase	1 - 5 WU
Nuclease-free water	to 20 µL

Incubate the reaction at 16 °C for 2-6 hours or 4 °C overnight.

2. Rapid Ligation
Assemble the following reaction in a sterile microcentrifuge tube. Example for a 1:1 vector:insert ratio:

Component	20 µL reaction
Ligase 2X Buffer	10 µL
Vector DNA (3 kb)	100 ng
Insert DNA (0.5 kb)	17 ng
T4 DNA Ligase	1 - 5 WU
Nuclease-free water	to 20 µL

Incubate the reaction at 16°C or room temperature for 20 minutes.