DATA SHEET

Jena Bioscience

T4 DNA Ligase

E. coli lambda lysogen NM 989

Cat. No.	Amount
EN-149S	400 Weiss units (80000 CE units)
EN-149L	5 x 400 Weiss units (5 x 80000 CE 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.

For *in vitro* use only!

Shipping: shipped on blue ice

Storage Conditions: store at -20 °C

Additional Storage Conditions: avoid freeze/thaw cycles

Shelf Life: 12 months

Form: liquid (Supplied in 10 mM Tris-HCl pH 7.4, 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 200 µg/ml BSA and 50 % [v/v] glycerol)

Concentration: 2.5 Weiss units/µl (500 CE units/µl)

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

Content:
Standard Ligation Buffer, 10x conc.
500 mM Tris-HCl pH 7.8 at 25 °C, 100 mM $MgCl_2$, 100 mM DTT, 10 mM ATP and 25 µg/ml BSA

Fast Ligation Buffer, 2x conc.
60 mM Tris-HCl pH 7.8 at 25 °C, 20 mM $MgCl_2$, 20 mM DTT, 2 mM ATP and 10 % PEG

component	EN-149S	EN-149L
T4 DNA Ligase	160 µl	5 x 160 µl
Standard Ligation Buffer, 10x conc.	1 ml	5 x 1 ml
Fast Ligation Buffer, 2x conc.	5 ml	5 x 5 ml

Heat inactivation:
T4 DNA Ligase can be inactivated by incubation at 65 °C for 10 minutes.

Note:
- One Cohesive-End Ligation Unit (CEU) is defined as the amount of enzyme required to give 50 % ligation of *Hin*d III fragments of λ 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. 200 CE units.
- 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 addition of PEG 4000 (10 % w/v final concentration) or hexamine chloride, or by reducing the ATP concentration to 50 µM.
- 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.

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Assay Set-Up:
Standard Ligation Assay

comp.	final amount/conc.	20 µl assay
Standard Ligation Buffer, 10x conc.	1x	2 µl
Vector/Insert DNA	100 ng - 1 µg	100 ng - 1 µg
T4 DNA Ligase	0.1 - 1 Weiss units	0.04-0.4 µl
PCR-grade Water	-	fill up to 20 µl

Incubate for 20 - 30 min at 16 °C for optimal ligation.

Fast Ligation Assay

comp.	final amount/conc.	20 µl assay
Fast Ligation Buffer, 2x conc.	1x	10 µl
Vector/Insert DNA	100 ng - 1 µg	100 ng - 1 µg
T4 DNA Ligase	0.1 - 1 Weiss units	0.04-0.4 µl
PCR-grade Water	-	fill up to 20 µl

Incubate for 5 min for cohesive-ended ligations or 15 min for blunt-ended ligations at ambient temperature (20 - 25 °C).