-Datasheet-

T4 DNA Ligase (NEW with FAST-LIGATION Buffer)

T 4 DNA Ligase high concentrated catalyzes the formation of a phosphodiester bond between juxtaposed 5´ phosphate and 3´ hydroxyl termini in duplex DNA or RNA.

Description *T4 DNA Ligase* is purified from E.coli strain harbouring the plasmid that directs the synthesis of T4 DNA ligase.T4 DNA Ligase catalyzes the formation of phosphodiester bonds between 5' phosphate and 3' hydroxyl termini in duplex DNA/RNA.T4 Ligase can join blunt and cohesive end termini, repair single strand nicks an duplex DNA, RNA, or DNA/RNA hybrids.

Concentration 2,5 Weiss-Units / µl (500 CE units/µl)

Source Purified from E. coli strain harbouring the plasmid that directs the synthesis of T4 DNA ligase.

Applications Cloning of restriction fragments, joining linkers and adapters to blunt-ended DNA, gene (gene fragments) synthesis.

Cohesive End Ligation: For most cohesive end ligations, a 30 minute incubation at 20°C is sufficient. Incubations at 16°C for 4-16 hours are routinely used for the majority of applications.

Ligation of blunt ends and single-base pair overhang fragments requires more enzyme to achieve the same extent of ligation as cohesive end DNA fragments. Ligation may be enhanced by addition of PEG, or by reducing the ATP concentration.

ATP is an essential cofactor for the reaction.

Storage buffer 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)

Unit definition One unit is defined as the amount of enzyme required to give 50% ligation of Hind III fragments of lambda DNA in 30 minutes at 16°C at 5' termini concentration of 0.12 µM (300 µg/ml).

Reaction buffer delivered (10X) 500 mM Tris HCl (pH 7,8), 100mM MgCl2, 100mM DTT, 10 mM ATP, 25 µg/µl BSA

Fast Ligation Buffer (2X): 60 mM Tris-HCl pH 7.8 at 25 °C, 20 mM MgCl2, 20 mM DTT, 2 mM ATP and 10 % PEG

Inactivation: 10 minutes at 65 °C

Quality Assurance Each lot of T4 DNA ligase is tested for absence endonucleases/exonucleases

Note:
• 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.

Storage conditions: Storage temperature is -20°C

.. a good decision ..

Protocol:

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 Buf fer, 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).

Ordering information

Cat.-no	Description	Amount
402-002	T 4 DNA Ligase	400 Weiss-units
402-010	T 4 DNA Ligase	2000 Weiss-units