GENAXXON bioscience
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T4 DNA Ligase

Component	Cat#	M3027.1010 10000 CEL	M3027.5010 50000 CEL	Colour code of cap
T4 DNA Ligase (166.68 CEL/µL ≡ 2.5 WU/µL)		60µL	300µL	colourless
10X T4 Ligase Buffer with ATP		380µL	5x 380µL	brown
2X FAST T4 Ligase Buffer with ATP		2x 960µL	10x 960µL	orange

Concentration: 166.68 CEL units/µL.

Description: T4 DNA Ligase catalyses the formation of phosphodiester bonds between adjacent 5' phosphate and 3' hydroxyl termini in duplex DNA/RNA. This enzyme can join blunt end and cohesive end termini, repair single stranded nicks in duplex DNA, RNA or DNA/RNA hybrids.

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

Unit Definition (CEL): One CEL 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 0.12mM (300µg/mL).

One Cohesive End Ligation Unit (CEL) equals 0.015 Weiss units.
One Weiss unit equals 67 Cohesive End Ligation units.

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 20 to 30-minute incubation at 16°C is sufficient.
Incubation at 16°C for 4 – 16 hours are routinely used for most applications.
Amount of enzyme used for ligation: 0.1 – 1 Weiss units or 6.67 up to 66.67 CEL units are used for "sticky-end" ligation in 20µL reaction volume.

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.
Amount of enzyme used for blunt-end ligation: Up to 330 CEL units are used for blunt-end ligation in 20µL reaction volume.

Inactivation of T4 Ligase: 10 minutes at 65°C

ATP is an essential co-factor for all reaction with T4 DNA Ligase

Storage buffer: 10 mM Tris-HCl (pH7.4), 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 200 µg/mL BSA in 50% glycerol.

Reaction buffer (10X): 500 mM Tris-HCl (pH7.8), 100 mM MgCl2, 100 mM DTT, 10 mM ATP, 25 µg/mL BSA

Fast Ligation buffer (2X): 60 mM Tris-HCl (pH7.8), 20 mM MgCl2, 20 mM DTT, 2 mM ATP, 10% PEG

Storage: Please store at -20°C

Quality Assurance: Each lot of T4 DNA Ligase is tested for endonucleases / exonucleases in a Blue / White screening assay.
98% protein homogeneity in a 10% SDS-PAGE.

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Protocols for T4 DNA-ligase

DNA insert ligation into vector DNA sticky-end ligation - Standard Ligation assay

Prepare the following mixture

Component	amount	20µL assay
Vector/Insert DNA	100ng to 1µg	100ng to 1µg
10X FAST T4 DNA Ligase Buffer	1x	2µL
T4 DNA Ligase	6.67 - 66.67 CEL units respective 0.1 - 1 Weiss units	0.04µL to 0.4µL
Nuclease-free water		Fill up to 20µL
Total volume		**20µL**

1. Mix, spin briefly and **incubate 20 - 30 min. at 16°C for optimal ligation**.

2. Use up to 5µL of the mixture for transformation of 50µL of chemically competent cells or 1-2µL per 50µL of electrocompetent cells.

3. Heat inactivate at 65°C for 10 min. or at 70°C for 5 min.

DNA insert ligation into vector DNA sticky-end ligation - Fast Ligation assay

Prepare the following mixture

Component	amount	20µL assay
Vector/Insert DNA	100ng to 1µg	100ng to 1µg
2X FAST T4 DNA Ligase Buffer	1x	10µL
T4 DNA Ligase	6.67 - 66.67 CEL units respective 0.1 - 1 Weiss units	0.04µL to 0.4µL
Nuclease-free water		Fill up to 20µL
Total volume		**20µL**

1. Mix, spin briefly and **incubate for 5 min. for cohesive-end ligations or 15 min. for blunt-end ligations at ambient temperature (20°C to 25°C)**.

2. Use up to 5µL of the mixture for transformation of 50µL of chemically competent cells or 1-2µL per 50µL of electrocompetent cells.

3. Heat inactivate at 65°C for 10 min. or at 70°C for 5 min.

NOTE:

T4 DNA Ligase is strongly inhibited by NaCL or KCl if their concentration exceeds 200mM.

Ligation of blunt-ended and single-base pair overhang fragments requires about 50-times as much enzyme to achieve the same extent of ligations 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 50µM.

To dilute T4 DNA Ligase for subsequent storage at -20°C the usage of a storage buffer containing 50% glycerol is recommended.

To dilute T4 DNA Ligase for immediate use, usage of 1X Reaction Buffer is recommended.