

T4 DNA Ligase

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Product	Cat#	Package size
T4-DNA-Ligase	M3027.2000	2000 CEL units
T4-DNA-Ligase	M3027.1010	10000 CEL units

Concentration: 50-100 CEL units/μL

Description : T4 DNA Ligase catalyses the formation of a phosphodiester bond between 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: 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 0.12mM (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 30 minute incubation at 20°C (RT) is sufficient. Incubation at 16°C for 4 - 16 hours are routinely used for the majority of applications. Amount of enzyme used for ligation: 1 - 2 Weiss units or 70 up to 140 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 PRG, 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.

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

Storage buffer: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 50% glycerol.

Reaction buffer (1X): 50 mM Tris-HCl (pH 7.8), 10 mM MgCl₂, 10 mM DTT, 1 mM ATP

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.