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DATA QUALITY SHEET

T4 DNA Ligase

T4 DNA Ligase catalyzes the formation of a phosphodiester bonds 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 duplexDNA, RNA or DNA/RNA hybrids.Purified from *E. coli* strain harbouring the plasmid that directs the synthesis of T4 DNA ligase.
Concentration 50 – 100 units/µl

Application

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

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 can be enhanced by addition of PEG or by reducing the rATP concentration.
ATP is an essential cofactor for the reaction.

Storage Buffer

- 50 mM KCl
- 10 mM Tris-HCl (pH 7.4)
- 0.1 mM EDTA
- 1 mM DTT
- 50% 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). One Cohesive-End Ligation Unit equals 0.015 Weiss units. One Weiss unit equals 67 Cohesive-End Ligation Units.
Reaction Buffer x1

- 50 mM Tris HCL (pH 7.5)
- 10 mM MgCl 2
- 10 mM DTT
- 1 mM ATP.

QC

Each lot of T4 DNA ligase is tested for endonucleases/exonucleases, in a blue/white cloning assay.

Inactivation Conditions

65 °C for 15 minutes or boiling for 2 minutes

Storage Conditions -20 °C