

E.coli DNA Ligase



Catalog: RK20501

Size: 200 U / 1,000 U

Concentration: 10,000 U/ml

Components:

<i>E.coli</i> DNA Ligase (10,000 U/ml)	RM20505
10X <i>E.coli</i> DNA Ligase Reaction Buffer	RM20128

Product Description

E.coli DNA Ligase catalyzes the formation of a phosphodiester bond between the 5'-phosphate and the 3'-hydroxyl of two adjacent DNA strands in duplex DNA with cohesive ends. It is not appreciably active on blunt-ended substrates. *E.coli* DNA Ligase uses NAD as a cofactor and can be heat-inactivated. *E.coli* DNA Ligase is active at a range of temperatures (4°C – 37°C).

Product Source: Purified from *E.coli* strain containing a cloned *E.coli* DNA Ligase gene.

Unit Definition: One unit is defined as the amount of enzyme required to give 50% ligation of HindIII fragments of λ DNA (5' DNA termini concentration of 0.12 μ M, 300 μ g/ml) in a total reaction volume of 20 μ l in 30 minutes at 16°C in 1X *E.coli* DNA Ligase Reaction Buffer.

Reaction Conditions:

1X *E.coli* DNA Ligase Reaction Buffer, Incubate at 16°C.

1X *E. coli* DNA Ligase Reaction Buffer:

30 mM Tris-HCl, 4 mM MgCl₂, 26 μ M NAD, 1 mM DTT, 50 μ g/ml BSA, pH 8 @ 25°C.

Storage Temperature: -20°C

Storage Conditions:

10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 200 μ g/ml BSA, 50 % Glycerol, pH7.4@25°C

Heat Inactivation: 65°C for 20 min

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Instructions

1. Set-up the reaction as follows:

H ₂ O	up to 20 μ l
10X <i>E.coli</i> DNA Ligase	2 μ l
Reaction Buffer	
DNA	up to 5 μ g
<i>E. coli</i> DNA Ligase	1 μ l (10 units)

2. Incubate at 16°C for 30 minutes.
3. Heat inactivate by incubating at 65°C for 20 minutes.

Notes:

1. Requires NAD⁺ (nicotinamide adenine dinucleotide) as a cofactor, in contrast to other ligases which use rATP.
2. Ligation of blunt-ended fragments is extremely inefficient. For ligation of blunt-ended fragments use T4 DNA Ligase.
3. Does not ligate RNA to DNA.
4. This enzyme ligates only DNA fragments with cohesive termini.

QC Process:

- ◆ Purity (SDS-PAGE) is above 95%.
- ◆ No endonucleases, ss-DNase, or other RNase contamination.
- ◆ No residual host genomic DNA detected by PCR.