E.coli DNA Ligase

Catalog: RK20501

Size: 200 U / 1,000 U

Concentration: 10,000 U/ml

Components:

E.coli DNA Ligase (10,000 U/ml)	RM20505
10X *E.coli* 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 ℃ – 37 ℃).

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 μM, 300 μg/ml) in a total reaction volume of 20 μl 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 ℃.

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

30 mM Tris-HCl, 4 mM $MgCl_2$, 26 μM NAD, 1 mM DTT, 50 μg/ml BSA, pH 8 @ 25°C.

Storage Temperature: -20 ℃

Storage Conditions:

10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 200 μg/ml BSA, 50 % Glycerol, pH7.4@25 ℃

Heat Inactivation: 65 ℃ for 20 min

Instructions

1. Set-up the reaction as follows:

H_2O	up to 20 μl
10X *E.coli* DNA Ligase Reaction Buffer	2 μl
DNA	up to 5 μg
E. coli DNA Ligase	1 μl (10 units)

2. Incubate at 16 ℃ for 30 minutes.
3. Heat inactivate by incubating at 65 ℃ 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 is above 95% detected by SDS-PAGE.
- No endonucleases, ss-DNase and other RNases contamination.
- Host genomic DNA is no residual detected by PCR.