

Product components

Components	Component number	Concentration	200 U	1,000 U
<i>E.coli</i> DNA Ligase	RM20505	10,000 U/mL	20 µL	100 µL
10X <i>E.coli</i> DNA Ligase Reaction Buffer	RM20128	10X	50 µL	250 µL

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.

Storage

E.coli DNA Ligase, store at -20°C.

10X *E. coli* DNA Ligase Reaction Buffer, -20°C for short term storage, -80°C for long-term storage.

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°C

1X *E. coli* DNA Ligase Reaction Buffer

30 mM Tris-HCl, 4 mM MgCl₂, 26 µM NAD, 1 mM DTT, 50 µg/mL BSA, pH 8 @ 25°C.

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°C

Heat Inactivation

65°C for 20 min

Instructions

1. Set up the reaction as follows.

Composition	Amount
ddH ₂ O	Up to 20 µL
10X <i>E. coli</i> DNA Ligase Reaction Buffer	2 µL
DNA	Up to 5 µg
<i>E. coli</i> DNA Ligase	1 µL (10 U)

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.
5. 10X *E. coli* DNA Ligase Reaction Buffer contains the cofactor NAD and should be stored at -80°C for a long time to prolong the half-life of NAD.