Tehran Cavosh Clon
Our Aim is Your Success

T4 DNA Ligase, 5 Weiss U/µl
For Research Use Only

Cat. No.: MO5454 **Store at**: -20°C
Concentration: 5 U/µl , **100 Weiss U**
Supplied with: 80 µl of 10X Reaction Buffer

Description

Purified from an *E.coli* strain carrying a plasmid with the cloned gene of phage T4 encoding this enzyme. 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 duplex DNA, RNA or DNA/RNA hybrids.

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.

Storage Conditions

50 mM KCl, 50 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM DTT, 50% glycerol at -20°C.

Application

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

Ligation

For most cohesive-end ligations, at least 60 minutes incubation at room temperature 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.

We recommend using a 1:1, 1:3 or 3:1 molar ratio of vector: insert DNA when cloning a fragment into a plasmid vector. These ratios will vary with other types of vectors, for example, cDNA and genomic cloning vectors. The following example illustrates the conversion of molar ratios to mass ratios for a 3.0kb plasmid and a 0.5kb insert DNA fragment.

ng of vector × kb size of insert × molar ratio of insert = ng of insert kb size of vector vector

Example:

How much 0.5 kb insert DNA should be added to a ligation in which 100ng of 3kb vector will be used? The desired vector: insert ratio will be 1:3.

$$\frac{100\ \text{ng vector} \times 0.5\ \text{kb insert}}{3\ \text{kb vector}} \times \frac{3}{1} = 50\ \text{ng insert}$$

The following ligation reaction of a 3kb vector and a 0.5 kb insert DNA uses a 1:1 vector: insert ratio. Typical ligation reactions use 100–200 ng of vector DNA.

Assemble the following reaction in a sterile microcentrifuge tube:

Vector DNA	variable
Insert DNA	variable
Ligase 10X Buffer	2 µl
T4 DNA Ligase	1 µl
Nuclease-Free Water to final volume of	20 µl

Inactivation Conditions

65 °C for 15 minutes or boiling for 2 minutes.

QC

tested for the absence of endo-exodeoxyribonucleases, ribonucleases.

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