Taq DNA Ligase

REF: EG20201S

Storage Condition

-20℃

Components

Component	Amount
Taq DNA Ligase (40 U/μl)	25 μl
10× Taq DNA Ligase Buffer	1 ml

Note: 1 U = 1 CEU

Description

Taq DNA Ligase is a thermostable ligase that catalyzes the formation of phosphodiester bonds between the 5'-phosphate and 3'-hydroxyl ends of adjacent oligonucleotide chains that hybridize to the same complementary target DNA strand. This reaction occurs only when both oligonucleotide chains are fully complementary to the target DNA and there are no gaps between them. Therefore, it can be used for single-base substitution detection, but not the substitute of T4 DNA ligase. Taq DNA Ligase is active at the temperature from 45 to 65℃ and requires NAD^+ as a coenzyme.

Definition of Activity Unit

One unit is defined as the amount of enzyme required to give 50% ligation of 1 μg of BstEII-digested λDNA in a total reaction volume of 50 μl in 15 minutes at 45℃ .

Quality Control Assays

Endonuclease Activity

A 50 μl reaction containing 200 ng of supercoiled plasmid and 80 U of Taq DNA ligase incubated for 4 hours at 37℃ results in <10% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

Non-specific Nuclease Activity

A 50 μl reaction containing 15 ng of dsDNA fragments and 80 U of Taq DNA ligase incubated for 16 hours at 37℃ results in no detectable degradation of the dsDNA fragments as determined by agarose gel electrophoresis.

Protocol

① Prepare the following reaction mixture on ice:

Reagent	Amount
DNA	up to 1 μg
10× Taq DNA Ligase Buffer	5 μl
Taq DNA Ligase	2 μl
ddH_2O	To 50 μl

② Mix gently and spin down, then incubate at 45℃ for 15 minutes;

③ Terminate the reaction by adding a termination buffer (50% glycerol, 50 mM EDTA, and bromophenol blue). Do not heat-inactivate the enzyme.

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