HiFi Taq DNA Ligase

REF: EG24203S

Storage Condition

-20℃

Components

Component	Amount
HiFi Taq DNA Ligase	50 rxns
10× HiFi Taq DNA Ligase Buffer	500 μl

Description

HiFi Taq DNA Ligase is a mutant type of Taq DNA ligase, which has higher ligation efficiency and high fidelity compared with the wild type. Meanwhile, it maintains the basic functions of the wild type and is a high-temperature-resistant ligase with activity in the range of 37~75℃ .Taq DNA ligase, with NAD^+ as the coenzyme factor, catalyzes the formation of a phosphodiester bond between the 5'-phosphate and 3'-hydroxyl groups of two adjacent oligonucleotide chains hybridized to the same complementary target DNA strand, and it has significant activity only for DNA nicks. This product is applicable to molecular diagnostic reactions that rely on nick junction, such as ligase detection reaction (LDR), multiplex ligation-dependent probe amplification (MLPA), etc.

Quality Control Assays

Endonuclease Activity

A 20 μl reaction containing 200 ng of supercoiled plasmid and 1 μl 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 1 μl 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.

RNase Activity

A 10 μl reaction containing 500 ng of RNA and 1 μl of HiFi Taq DNA Ligase incubated for 1 hour at 37℃ results in >90% of the substrate RNA remains intact as determined by agarose.

Residual Host DNA

The product was tested by qPCR with primers specific for the E.coli 16S rDNA , and the results show that the *E.coli* genome residues less than 10 copies/μl。

Recommended reaction system

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

Recommended reaction condition

Incubate at 45~65℃ for 15 min.

Notice

1. Reaction conditions: The reaction temperature range of HiFi Taq DNA Ligase is 37~75℃ . For a given set of probes, the optimal ligation incubation temperature is usually within 5℃ of the Tm value of the probe annealing region. At the same time, in practical applications, the best balance between activity and fidelity needs to be achieved based on experience.

2. For classic LDR detection, the reaction time is usually between 10~60 min, and a 15-min reaction time is suitable for most applications. For detections that require denaturation/annealing/ligation cycles, we recommend denaturation at 95℃ for 30 s~2 min, and then annealing/ligation at the optimized ligation temperature of the probe group for 1~5 min.

3. The 10× HiFi Taq DNA Ligase Buffer contains NAD^+ as a cofactor. Aliquot it and store it at -80℃ to further extend the half-life of the NAD^+ cofactor. After the reaction Buffer is thawed from low temperature to room temperature, white precipitates may appear in the solution. You can pipette it 20 to 30 times or vortex it to dissolve the precipitate before use. If the reaction buffer turns brown, it can still be used.

4. When observing the ligation reaction by gel electrophoresis, it is recommended to use Proteinase K for product treatment before electrophoresis to avoid gel retardation.

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