Tinzyme Co., Limited

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Hifi Taq Ligase

Product Number: LG04

Shipping and Storage

Hifi Taq Ligase stored at -20℃ and buffer at -70℃.

Components

Component	LG04	LG04	LG04
Hifi Taq Ligase (40U/μl)	25μl	50μl	1ml
10×Taq Ligase Reaction Buffer	500μl	1ml	10ml×2

Description

This enzyme is a DNA ligase cloned from Thermos aquaticus HB8, expressed in Escherichia coli using gene recombination technology and purified multiple times.Hifi Taq Ligase can catalyze the formation of phosphodiester bonds, connecting the 5'-phosphate end 3' - hydroxyl end of two compatible oligonucleotide chains hybridized to the same complementary target DNA strand through phosphodiester bonds.This connection reaction can only occur when two oligonucleotide chains are completely paired with complementary target DNA and there is no gap between the two oligonucleotide chains. Therefore, it can be used to detect single base substitution. Hifi Taq Ligase is active within the range of 45-65℃.

Features

1. Good heat resistance.
2. Active over a wide temperature range.

Application

1. Mutation was performed by PCR amplification by incorporating phosphorylated oligonucleotides.
2. Use ligase detection reaction and ligase chain reaction for specific detection of alleles.
3. Detect single base substitution.

Unit definition

In a 50μl reaction system, the amount of enzyme required to connect 50% of the 12bp sticky end fragments can be achieved within 15 minutes under 45℃ reaction conditions. 12bp sticky end from BstEII digestion 1μg λDNA.

Quality control

After multiple column purification, only a clear and single target band can be seen in SDS-PAGE gel detection. Strict quality control ensures that the product has the highest activity and purity.

Protocol

1. Common reaction systems(50μl)

DNA	up to 1 μg
10×Taq Ligase Reaction Buffer	5μl
Hifi Taq Ligase (40 U/μL)	2μl
ddH_2O	up to 50μl

2. Reaction conditions: Incubate at 45℃ for 15 minutes. Add termination dye solution (50% glycerol, 50mM EDTA, and bromophenol blue) to terminate the reaction.

Note:1)This enzyme requires NAD+ as a cofactor, 10×Taq Ligase Action Buffer already contains NAD+, and the buffer should

be stored at -70℃ to extend the half-life of NAD+.

2)1×Taq Ligase Reaction Buffer: 20mM Tris-HCl (pH7.6,25℃), 25mM KAc, 10mM $Mg(Ac)_2$,10mM DTT, 1mM NADand 0.1% Triton X-100,45℃ incubation.

3)Hifi Taq Ligase cannot replace T4 DNA ligase.