

Product components

Components	Component number	Concentration	Size1 (10,000 U)	Size2 (10,000 U)	Size3 (40,000 U)
Taq DNA Ligase	RM20506	40,000 U/mL	50 µL	250 µL	1 mL
10X Taq DNA Ligase Reaction Buffer	RM20131	10X	150 µL	650 µL	1.25 mL X2

Product Description

Taq DNA Ligase is a thermostable ligase that catalyzes the formation of a phosphodiester bond between the 5´-phosphate and the 3´-hydroxyl of two adjacent DNA strands. The target strands need to be hybridized and accurately paired, with no gap, to a complementary DNA strand; allowing resolution of single nucleotide variants. Taq DNA Ligase uses NAD as a cofactor and it is active at elevated temperatures (37°C–75°C).

It is applicable to Allele-specific gene detection using Ligase Detection Reaction and Ligase Chain Reaction, as well as Mutagenesis by incorporation of a phosphorylated oligonucleotide during primer extension amplification.

Product Source

Purified from an *E.coli* strain containing the cloned ligase gene from *Thermus thermophilus* HB.

Storage

Taq DNA Ligase, store at -20°C.

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

Unit Definition (Cohesive End Unit)

One unit is defined as the amount of enzyme required to give 50% ligation of the 12-base pair cohesive ends of 1 µg of BstEII-digested λ DNA in a total reaction volume of 50 µl in 15 minutes at 45°C.

1X Taq DNA Ligase Reaction Buffer

20 mM Tris-HCl, 25 mM Potassium Acetate, 10 mM Magnesium Acetate, 1 mM NAD,

10 mM DTT, 0.1% Triton X-100, pH 7.6@25°C

Storage Conditions

10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, pH 7.4 @ 25°C

Heat Inactivation

No

Instructions

1. Set up the reaction as follows.

Composition	Amount
ddH ₂ O	Up to 50 µL
10X Taq DNA Ligase Reaction Buffer	5 µL
DNA	Up to 1 µg
Taq DNA Ligase	2 µL (80 U)

2. Incubate at 45°C for 15 minutes.

Notes

1. After thawing 10X Taq DNA Ligase Reaction Buffer (RM20131) from low temperature to room temperature, white precipitates may appear in the solution, which can be dissolved by blowing 20-30 times with a pipiter or by vortexing.
2. Reaction Conditions: Incubate DNA and enzyme in 1X Taq DNA Ligase Buffer at 45°C for 15 minutes or in a thermocycler with a program suited to the reaction described by [Barany (1991) *Genetic Disease Detection and DNA Amplification Using Cloned Thermostable Ligase. Proc. Natl. Acad. Sci. USA* 88, 189-193.]The reaction is stopped with a mixture of 50% glycerol, 50 mM EDTA, bromphenol blue.
3. 10X Taq 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.