

Catalog #	3218	3219
Package Size	2,000 units	10,000 units
Volume	50 µl	250 µl
Concentration	40 units/µl	

# Description

Tag DNA Ligase catalyzes the formation of a phosphodiester bond in duplex DNA containing adjacent 5'-phosphoryl and 3'-hydroxyl termini, using NAD+ as a cofactor. The ligation will occur only if the oligonucleotides are perfectly paired to the complementary target DNA and have no gaps between them; therefore, a single-base substitution can be detected. Tag DNA Ligase is active at elevated temperatures (45°C-70°C) (1, 2).

# **Protein Purity**

The physical purity of this enzyme is  $\geq$ 99% as assessed by SDS-PAGE with Coomassie® blue staining (see figure below).



Figure: Lane 1. Protein Marker Lane 2. Taq DNA Ligase

# Product Source

E. coli strain expressing the cloned Tag DNA ligase gene from Thermus agauticus HB8

# Applications

- Allele-specific gene detection by using Ligase Detection Reaction (LDR) and Ligase Chain Reaction (LCR)<sup>(1)</sup>.
- Mutagenesis by incorporation of a phosphorylated oligonucleotide during primer extension amplification (3).

# Product Includes

- 1) Tag DNA Ligase
- 2) 10X Tag DNA Ligase Buffer with NAD+

#### **Storage Temperature**

-20 °C

# Storage Buffer

50 mM Tris-HCI, 50 mM KCI, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, pH 7.5 @ 25 °C

#### 10X Tag DNA Ligase reaction buffer with NAD+

500 mM Tris-HCl, 100 mM MgCl<sub>2</sub>, 100 mM DTT, 10 mM NAD+, pH 7.5 @ 25°C

#### **Unit Definition**

One unit is defined as the amount of Tag DNA Ligase required to join 50% of 1 µg of the 12-base cohesive ends of Lambda DNA cut with Sma I and Sal I in 50 µl reaction in 15 min incubation at 45 °C.

# **Quality Control**

Tag DNA Ligase is free from detectable RNase or contaminating DNA endonuclease activities.

#### Protocol

1. Set-up the reaction as follows:

DNA	x µl (up to 1 µg)
10X Taq DNA Ligase Buffer	5.0 µl
Taq DNA Ligase	2.0 µl
H <sub>2</sub> O up to	50.0 µl

Incubate at 50 °C for 15-30 minutes. 2

#### References

- 1. Barany, F. (1991). Proc. Natl. Acad. Sci. USA. 88, 189-193.
- 2. Takahashi, M. et al. (1984). J. Biol. Chem. 259, 10041-10047.
- 3. Michael, S.F. (1994). Biotechniques. 16, 411-412.