For research use only

ISO9001

T4 DNA Ligase

Product	Quantity	Cat. No.	Remarks
T4 DNA Ligase	200 unit	EBT-1025	2 unit/㎕

Description

T4 DNA Ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5'-phosphate and 3'-hydroxyl termini in duplex DNA or RNA. This enzyme will join blunt end and cohesive end termini as well as repair single-stranded nicks in duplex DNA, RNA or DNA/RNA hybrids. T4 DNA Ligase is purified from a recombinant *E.coli* strain.

Reaction Conditions

Incubate at 16-37°C in 1 x T4 DNA ligase reaction buffer.

5x T4 DNA Ligase Reaction Buffer

150mM Tris-HCl, pH 7.8, 50mM $MgCl_2$, 5mM ATP, 50 mM DTT.

Unit Definition

0.01 Weiss unit of enzyme is defined as the amount of enzyme required to give 90% ligation of 1 μg Hind III fragments of lambda DNA in 30 min at 16°C in 20 μl of the assay mixture.

Concentration

2 unit/μl

Storage Conditions

10 mM Tris-HCl, pH 7.4, 50mM KCl, 1mM DTT, 0.1mM EDTA, 50% glycerol

QC Tests

Activity, exo and endonuclease activity test, SDS-PAGE purity, performance tests.

Storage Condition

Store at -20℃.

Heat Inactivation

T4 DNA Ligase can be inactivated by incubation at 65°C for 10 min.

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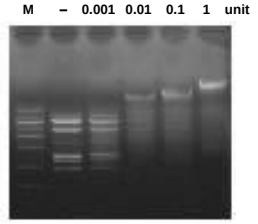
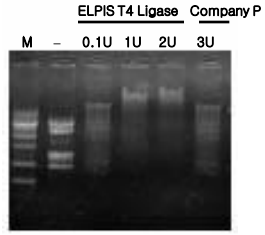


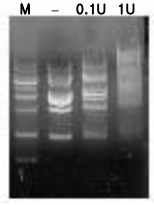
Unit determination

One μg of Hind III digested DNA in 10 μl reaction mixture
Reaction temperature : 16°C
Reaction time : 30 min
T4 DNA ligase : 0.001 to 1 units



Efficiency test for cohesive end ligation

One μg of Hind III digested DNA in 10 μl reaction mixture
Reaction temperature : room temperature (26°C)
Reaction time : 5 min
T4 DNA ligase : 0.1 to 2 units compared with competitor's 3 units



Efficiency test for blunt end ligation

One μg of Sma I digested DNA in 10 μl reaction mixture
Reaction temperature : room temperature (26°C)
Reaction time : 30 min
T4 DNA ligase : 0.1 to 1 units

Quick Protocol for Ligation

1. Prepare ligation mixture as follows

   Vector DNA : 50 ng – 200 ng
   Insert DNA : insert : vector = 3 : 1 ratio
   5x ligation buffer : 2 μl
   T4 DNA ligase : 0.05 μl – 0.5 μl (0.1 –1 units for cohesive ends)
   0.5 μl – 1μl (1 –2 units for blunt ends)
   Adjust volume to 10 μl with D.W.

2. Incubate at 16°C for 30 min to 2 hours or at 20-25°C for 10 min (for cohesive end ligation).
   - For blunt end ligation, incubate at 20-25°C for 2 hours.

3. Transform *E.coli*. competent cells.

Caution: Rapid ligation with high concentration of T4 DNA ligase at elevated temperature may cause a concatemerization of vector and insert DNA, which decrease in overall transformation efficiency. In case, confirm appropriate ligation by agarose gel electrophoresis before transformation and decrease amount of T4 DNA ligase added to ligation mixture. 0.1 unit of T4 DNA ligase is sufficient for complete ligation of conventional cohesive end at 16°C for 30 min