Invitrogen
life technologies

T4 DNA Ligase

Cat. No. 15224-041 **Size: 250 units**

Conc.: 5 U/µl

Store at -20°C in a non- frost-free freezer.

Note: T4 DNA Ligase is unstable on ice for long periods. Therefore, Invitrogen recommends the enzyme be kept at -20°C until within 5-10 minutes of use and returned IMMEDIATELY to -20°C after use.

Description:

T4 DNA Ligase can be used to join DNA fragments with staggered or blunt ends and to repair nicks in double-stranded DNA having 3'-hydroxyl and 5'-phosphate ends. The enzyme is isolated from *E. coli* lambda lysogen NM989.

Unit Definition:

One (Weiss) unit catalyzes the exchange of 1 nmol ^{32}PPi into [γ/β-^{32}P]ATP in 20 minutes at 37°C (1).

Components:

T4 DNA Ligase
5X DNA Ligase Reaction Buffer

Buffer Composition:

T4 DNA Ligase Storage Buffer:
10 mM Tris-HCl (pH 7.5)
50 mM KCl
1 mM DTT
50% (v/v) glycerol

5X DNA Ligase Reaction Buffer:
250 mM Tris-HCl (pH 7.6)
50 mM $MgCl_2$
5 mM ATP
5 mM DTT
25% (w/v) polyethylene glycol-8000.
Store buffer at -20°C.

Doc. Rev.: 051502

Quality Control:

This product has passed the following quality control assays: functional absence of endonuclease and exonuclease activities: ligation/recut and ligation efficiency.

The enclosed buffers were assayed with the enzyme and met quality control specifications.

Protocols:

Note: Before use, thaw 5X DNA Ligase Reaction Buffer at room temperature and vortex vigorously to dissolve any precipitated material.

Recommended Conditions for General Cloning and Library Construction:

	Cohesive Ends	Blunt Ends
5X Ligase Reaction Buffer	4 µl	4 µl
Insert: Vector Molar Ratio	3:1	3:1
Vector Ends	3-30 fmol	15-60 fmol
Insert Ends	9-90 fmol	45-180 fmol
Total DNA	0.01-0.1 µg	0.1-1.0 µg
T4 DNA Ligase	0.1 unit	1.0 unit
Autoclaved distilled water	to 20 µl	to 20 µl
Temperature	23-26°C	14°C
Time	1 h	16-24 h

Note: For optimal transformation, dilute the ligation reaction ≥ 5-fold, to at least 100 µl, before adding to competent cells (2).

Rapid Ligation (5-min) Protocol for Plasmid Cloning of DNA Fragments:

A molar ratio of 3:1 insert:vector is recommended for the rapid ligation of DNA inserts to vectors to produce circular recombinant molecules. Subsequent to restriction endonuclease digestion, purify the insert DNA from agarose using the S.N.A.P.™ Gel Purification Kit. Following restriction endonuclease digestion, dephosphorylate the vector DNA. Dephosphorylated vector can be used without purification if Calf Intestinal Alkaline Phosphatase (CIAP) is heat-inactivated prior to ligation.

1. To an autoclaved, 1.5-ml microcentrifuge tube, add the following:

	Cohesive Ends	Blunt Ends
5X Ligase Reaction Buffer	4 µl	4 µl
Vector DNA	3 to 30 fmol	15 to 60 fmol
Insert DNA	9 to 90 fmol	45 to 180 fmol
T4 DNA Ligase (units)	1 unit (in 1 µl)*	5 units (in 1 µl)
Autoclaved distilled water	to 20 µl	to 20 µl

*Dilute the T4 DNA Ligase supplied in the kit (5U/µl) using the storage buffer solution described on page 1.

1. Mix gently. Centrifuge briefly to bring the contents to the bottom of the tube.
2. Incubate at room temperature for 5 min.
3. Use 2 µl of the ligation reaction to transform 100 µl of MAX Efficiency® Competent cells.

References:

1. Weiss, B., Jacquemin-Sablon, A., Live, T.R., Fareed, G.C., and Richardson, C.C. (1968). *J. Biol. Chem.* 243, 4543.
2. Jesse, J. (1984). *Focus* ® 6:4.