

Product Insert

T4 DNA Ligase

Catalogue Number:

BIO-27026 500 Units

Features

- Catalyzes the joining of double-stranded DNA
- Supplied with 10x reaction buffer and ATP
- No loss of transformation efficiency

Applications

- Ligation of cohesive and blunt-ended DNA fragments for cloning
- Sealing nicks in double-stranded DNA
- Ligation of synthetic linkers to blunt-ended DNA

Description

T4 DNA Ligase catalyzes the joining of two strands of DNA between the 5'-phosphate and the 3'-hydroxyl groups of adjacent nucleotides in either a blunt-ended or cohesive-ended configuration. T4 DNA Ligase catalyzes the joining of RNA to either DNA or RNA strands in a duplex molecule but will not join single-stranded nucleic acids. T4 DNA Ligase is ATP dependent.

Recommendations

It is recommended that the ATP solution provided is aliquoted into smaller vials that can be defrosted when required. Repeated freeze/thawing of this solution is not recommended.

Components

	500 Units
T4 DNA Ligase	100µl
10x Reaction Buffer	1.2ml
ATP Solution	1.2ml

Reagent Specifications:

10x Reaction Buffer: 660 mM Tris-HCl (pH 7.6), 50 mM MgCl₂, 100 mM DTT.
Separate ATP Solution: 10 mM ATP in 50 mM Tris-HCl (pH 7.5).

Reaction Conditions (for a 20µl volume)

10x T4 Ligase Reaction Buffer	2.0µl
10x ATP Solution	2.0µl
Vector	as required
Insert	as required
Enzyme	1.0-1.5µl
Water (ddH ₂ O)	up to 20µl

For blunt ended DNA, run the DNA ligation for 1-2 hours at room temperature. For 3'-dA ends, incubate the reaction for 16-24 hours at 12-14°C.

The Total DNA concentration should be no more than 100ng.

The recommended **Insert : Vector** ratio should be between 6 and 2. A smaller ratio will result in a less efficient ligation, whilst a higher ratio will incite multiple insertions.

This data is intended for use as a guide only; conditions will vary from reaction to reaction and may need optimization.

Product Specifications

Batch details:

Batch No: See vial
Units per vial: See vial
Concentration: 5u/µl

Storage and Dilution Buffer:

10 mM Tris-HCl, pH 7.4, 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA and 50% glycerol.

Storage Conditions:

T4 DNA Ligase can be stored at -20°C in a constant temperature freezer for 12 months.

Shipping Conditions:

On Blue Ice

Q/C Assay Conditions:

Complete ligation of cohesive-ended *Hind* III fragments was achieved using only 0.01 units of enzyme per mg of Lambda DNA when incubated for 30 minutes at 16°C. Efficient ligation of blunt-ended *Hae* III fragments was achieved using 1.0 units of enzyme per mg of DNA when incubated for 30 minutes at 16°C.

Unit Definition:

One Weiss unit is defined as the amount of enzyme required to catalyze the conversion of 1nmole of ³²P pyrophosphate into Norit-adsorbable material in 66mM Tris HCl (pH 7.6), 6mM MgCl₂, 1mM DTT, 0.066mM ATP and 3.3mM [³²PPi] at 37°C for 20 minutes (2). 0.014 Weiss unit of ligase is equivalent to 1 ligation unit which catalyzes greater than 95% ligation of 1mg of Lambda/*Hind* III fragments at 16°C in 20 minutes.

Associated Products:

Product Name	Pack Size	Cat. No.
Quick-Stick Ligase	50 Reactions	BIO-27027
X-GAL	1g	BIO-37035
α-Select Bronze Competent Cells	2ml (10 x 200µl)	BIO-85025
BIOBlue Competent Cells	1ml (10 x 100µl)	BIO-85036