T4 DNA Ligase (Cloned)

Store at –20°C.
Do not store in a frost-free freezer.

Catalog #:	AM2134
Product Description:	High-purity T4 DNA Ligase, for use in ligation of blunt- and cohesive-ended DNA, and for nick repair in duplex DNA.
Source:	An *E. coli* strain overexpressing T4 DNA Ligase.
Amount:	1000 Units
Unit Concentration:	5 U/µL (Weiss)
Unit Definition:	One unit catalyzes the exchange of 1 nmol of inorganic phosphate from pyrophosphate into Norit-adsorbable material in 20 min at 37°C.
Additional Materials Included:	500 µL 10X T4 DNA Ligase Reaction Buffer 500 mM Tris, pH 7.8 100 mM $MgCl_2$ 100 mM DTT 10 mM ATP 0.25 mg/mL BSA *Note: Precipitates may form during storage; warm to 37°C and vortex to resuspend any precipitated material.*
Storage Conditions:	Store at –20°C. ***Do not store in a frost-free freezer.***
Storage Buffer:	(Not included) 20 mM Tris-HCl (pH 7.6), 60 mM KCl, 1 mM DTT, 1 mM EDTA, 50% glycerol (v/v).

USER INFORMATION

General Information:

When incubated in the presence of ATP (included in the supplied 10X Reaction Buffer), T4 DNA Ligase (E.C. 6.5.1.1) catalyzes the formation of phosphodiester bonds between the 5'-phosphoryl group and the 3'-hydroxyl group of double-stranded DNA fragments. T4 DNA Ligase will also close single-stranded "nicks" in double-stranded DNA molecules.

Applications:

The following general protocol is adapted from *Current Protocols in Molecular Biology*.

Mix together:
5 µL 10X T4 DNA Ligase Reaction Buffer (included)
1 µg DNA
1–5 U T4 DNA Ligase
To 50 µL with nuclease-free water

For cohesive (sticky) end ligations, incubate at room temperature for 1–3 hr. If poor results are obtained with a 1–3 hr incubation, incubating overnight at 16°C may improve the ligation reaction.

For blunt end ligation, incubate overnight at 16°C.

The reaction mixture can be used for transformation without further processing.

Reference:

Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K, editors. (2006) Enzymatic Manipulation of DNA and RNA (Chapter 3). In *Current Protocols in Molecular Biology*. John Wiley & Sons, Inc.

QUALITY CONTROL

T4 DNA Ligase is tested for contaminating nonspecific endonuclease, exonuclease, and protease activity. Functionally is determined in a ligation reaction with HindIII-cut Lambda DNA.

OTHER INFORMATION

Material Safety Data Sheets:

Material Safety Data Sheets (MSDSs) can be printed or downloaded from product-specific links on our website at the following address: www.ambion.com/techlib/msds. Alternatively, e-mail your request to MSDS_Inquiry_CCRM@appliedbiosystems.com. Specify the catalog or part number(s) of the product(s), and we will e-mail the associated MSDSs unless you specify a preference for fax delivery. For customers without access to the internet or fax, our technical service department can fulfill MSDS requests placed by telephone or postal mail. (Requests for postal delivery require 1–2 weeks for processing.)

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