



Product Information	
T4 DNA Ligase	
Part Number:	L603-LC-L
Lot Number:	

Rev. C

Product Description:

T4 DNA Ligase catalyzes the formation of a phosphodiester bond between the terminal 5' phosphate and a 3' hydroxyl groups of duplex DNA or RNA. The enzyme efficiently joins blunt and cohesive ends and repairs single stranded nicks in duplex DNA, RNA or DNA/RNA hybrids (1).

Source of Protein

A recombinant *E. coli* strain carrying the cloned T4 DNA Ligase gene.

Supplied In

10 mM Tris-HCl
50 mM NaCl
1 mM dithiothreitol
0.1 mM EDTA
50% glycerol
pH 7.5 @ 25°C

Supplied With

B603 (10X T4 DNA Ligase Buffer)

10X T4 DNA Ligase Buffer (B603)

500mM Tris-HCl
100 mM MgCl₂
50 mM dithiothreitol
10 mM ATP
pH 7.6 @ 25°C

Product Specification*

Unit Size	150,000 Units
Unit Concentration	120,000 U/mL
Volume	1.25 mL
Purity (SDS-PAGE)	>99%
Specific Activity	300,000 U/mg
SS Exonuclease	6,000 U <0.1% released
DS Exonuclease	6,000 U <0.1% released
Endonuclease	6,000 U <0.1% converted
<i>E. coli</i> 16S rDNA Contamination	3,000 U <10 copies
Storage	-20°C

* For a detailed summary of assay conditions and data, refer to the Quality Controls Analysis section below.

Molecular Weight

55.3 kDa

Unit Definition

1 unit is defined as the amount of T4 DNA Ligase required to join 50% of 100 ng of DNA fragments with cohesive termini in 50 µl 1X T4 DNA Ligase Buffer following a 30 minute incubation at 23°C.

Quality Control Analysis:

Unit Characterization Assay

Unit activity was measured using a 2-fold serial dilution method. Dilutions of enzyme batch were made in 1X T4 DNA Ligase Reaction Buffer ([T4 DNA Ligase]_i = 0.31-20 µg/µL) and added to 50 µL reactions containing 0.1 µg DNA and 1X T4 DNA Ligase Reaction Buffer. Reactions are incubated for 30 minutes at 23°C, stopped, and analyzed on a 1% agarose gel stained with ethidium bromide.

SDS-Page (Physical Purity Assessment)

2.0 µL of concentrated enzyme solution was loaded on a denaturing 4-20% Tris-Glycine SDS-PAGE gel flanked by a broad-range MW marker and 2.0 µL of a 1:100 dilution of the same enzyme species. Following electrophoresis, the gel was stained and the samples compared to determine physical purity. The acceptance criteria for this test requires that the aggregate mass of contaminant bands in the concentrated sample do not exceed the mass of the protein of interest band in the dilute sample, confirming greater than 99% purity of the concentrated sample.

Nuclease Contamination Tests:

Single-Stranded Exonuclease Activity

A 50 μ l reaction containing 15,000 cpm of a radiolabeled single-stranded DNA substrate and 10 μ L of enzyme solution incubated for 4 hours at 37°C resulted in less than 0.1% release of TCA-soluble counts.

Double-Stranded Exonuclease Activity

A 50 μ l reaction containing 10,000 cpm of a radiolabeled double-stranded DNA substrate and 10 μ L of enzyme solution incubated for 4 hours at 37°C resulted in less than 0.1% release of TCA-soluble counts.

Endonuclease Activity

A 50 μ L reaction containing 1 μ g of pENZuC DNA and 10 μ L of enzyme solution incubated for 4 hours at 37°C resulted in no visually discernible conversion to nicked circular DNA as determined by agarose gel electrophoresis.

Real-Time PCR DNA Contamination Test:

Replicate 5 μ L samples of enzyme solution were denatured and screened in a TaqMan qPCR assay for the presence of contaminating *E.coli* genomic DNA using oligonucleotide primers corresponding to the 16S rRNA locus. The acceptance criterion for the test is the threshold cycle count (C_t) produced by the average of 3 replicate no template control samples. Based on the correlation between the no template control C_t values and standard curve data, the detection limit of this assay is <10 copies genome/sample.

Notes

One Enzymatics T4 DNA Ligase cohesive end unit is equivalent to approximately 3 cohesive end units as measured with a Lambda-Hind III DNA fragment substrate in 1X T4 DNA Ligase reaction buffer.

One Weiss Unit is approximately equivalent to 22 Enzymatics cohesive end units.

For sticky end ligations, add 1 μ L T4 DNA Ligase to a final volume of 20 μ L and incubate at 25°C for 10 minutes.

Blunt-end ligations with Enzymatics' low-concentration T4 DNA Ligase (L603-LC-L) may be performed with 1 μ L T4 DNA Ligase in a final volume of 20 μ L and incubated at 25°C for 2 hours. Alternately, blunt end ligations can be performed in 10 minutes using Enzymatics' high concentration T4 DNA Ligase (L603-HC-L) and Rapid Ligation Buffer.

References

1. Engler, M.J. and Richardson, C.C. (1982) P.D. Boyer (Eds.), *The Enzymes*, 5, pp. 3. San Diego: Academic Press.



Limitations of Use

This product was developed, manufactured, and sold for *in vitro* use only. The product is not suitable for administration to humans or animals. MSDS sheets relevant to this product are available upon request.