Product Information

T7 DNA Ligase

Part Number:	L602L
Lot Number:	

Rev. B

Product Specification*

Unit Size:	900,000 Units
Unit Concentration	3,000,000 U/mL
Volume:	0.3 mL
Purity (SDS-PAGE)	>99%
Specific Activity	3,000,000 U/mg
SS Exonuclease	30,000 U <0.1% released
DS Exonuclease	30,000 U <0.1% released
Endonuclease	30,000 U <0.1% converted
E.coli 16S rDNA Contamination	30,000 U <10 copies
Storage	-20°C

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

Product Description:

T7 DNA Ligase catalyzes the formation of a phosphodiester bond between a 5′ phosphate and a 3′ hydroxyl termini in duplex DNA. The enzyme will join blunt end and cohesive end termini as well as repair single stranded nicks in duplex DNA.

Source of Protein

A recombinant *E. coli* strain carrying the T7 DNA Ligase gene.

Supplied in

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

Supplied With:

B101 (2X Rapid Ligation Buffer)

2X Rapid Ligation Buffer (B101):

132mM Tris-HCI
20 mM $MgCl_2$
2 mM dithiothreitol
2 mM ATP
15% PEG 8000
pH 7.6 @ 25°C

Unit Definition

1 unit is defined as the amount of T7 DNA Ligase required to ligate 50% of 100 ng DNA fragments with cohesive termini in 30 minutes 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 Rapid Ligation Buffer ($[\text{T7 DNA Ligase}]_f$ = 0.23-15 µg/µL) and added to 50 µL reactions containing 0.1 µg DNA and 1X Rapid Ligation Buffer. Reactions were incubated 30 minutes at 23ºC (room temp), plunged on ice, and analyzed on a 1% agarose gel stained with ethidium bromide. 1 unit is defined as the amount of T3 DNA Ligase required to ligate 50% of 100 ng DNA fragments with cohesive termini in 50 µL following a 30 minute incubation at 23°C.

Protein Concentration (OD_{280}) Measurement

A 3.0 µL sample of enzyme was analyzed at OD_{280} using a Nanodrop ND-1000 spectrophotometer standardized using a 2.0 mg/ml BSA sample (Pierce Cat #23209) and blanked with product storage solution. The observed average measurement of 3 replicate samples was converted to mg/mL using an extinction coefficient of 68,210 and molecular weight of 41,132 Daltons. Acceptance for this assay is +/- 5% of reference sample.

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 sample. 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.

(additional information on reverse side)

Nuclease Contamination Tests:

Single-Stranded Exonuclease Activity

A 50 µL reaction containing 20,000 cpm of a radiolabeled single-stranded DNA substrate and 10 µL 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 µL reaction containing 15,000 cpm of a radiolabeled double-stranded DNA substrate and 10 µL 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 µL reaction containing 1 µg of pBR322 DNA and 10 µL 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.

E.coli 16S rDNA Contamination Test

Replicate 5 µL samples of enzyme solution were heat denatured and screened in a TaqMan qPCR assay for the presence of contaminating *E.coli* genomic DNA using primers for the 16S rRNA locus. Based on no template control C_t values, the detection limit of this assay is <10 copies genome/sample.

Notes:

1. In the absence of high concentration crowding agents (eg 20-30% PEG 6-8000), the specific activity of T7 DNA Ligase is reduced by 1000-fold on blunt-ended fragments (1, 2).
2. T7 DNA Ligase is incapable of ligating single-stranded DNA fragments (1).

References:

1. Doherty, A. et al. J.Biol. Chem (1996) V.271, No.19, 11083-11089.
2. Dayal, T. Unpublished observations.

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.

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