

Product Information	
T3 DNA Ligase	
Part Number	L6010L 280210
Price	
Concentration	3,000,000 U/mL
Unit Size	900,000 U

### Product Description:

T3 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. In the absence of 20-30% PEG 6000, T3 DNA Ligase displays a very low efficiency for blunt-ended ligation. (1) T3 DNA Ligase displays a higher efficiency for joining A/T overhangs than C/G matched ends. (1) T3 DNA Ligase retains 95% of its activity in 1.0 M NaCl or KCl, with an optimal concentration of 300 mM(1).

### Source of Protein

A recombinant *E. coli* strain carrying the T3 DNA Ligase gene.

### Supplied in

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

### Supplied with

B1010 (2X Rapid Ligation Buffer)

### 2X Rapid Ligation Buffer (B1010):

132mM Tris-HCl  
20 mM MgCl<sub>2</sub>  
2 mM DTT  
2 mM ATP  
15% PEG 6000  
pH 7.6

## Product Information Sheet

Product Specification	
Storage Temperature	-25°C to -15°C
TEST:	SPECIFICATION:
Purity (SDS-PAGE)	>99%
Specific Activity	3,000,000 U/mg
SS Exonuclease	30,000 U <1.0% released
DS Exonuclease	30,000 U <1.0% released
DS Endonuclease	30,000 U =No conversion
<i>E.coli</i> DNA Contamination	30,000 U <10 copies

### Unit Definition

1 unit is defined as the amount of T3 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 ([T3 DNA Ligase]<sub>f</sub> = 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 23°C (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<sub>280</sub>) Measurement

A 2.0 µL sample of enzyme was analyzed at OD<sub>280</sub> 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 60,880 and molecular weight of 39,350 Daltons. Acceptance for this assay is +/- 5% of reference sample.

### SDS-Page (Physical Purity Assessment) and Specifications

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.

### **Contamination Tests:**

#### **Single-Stranded Exonuclease Activity**

A 50 µL reaction containing 10,000 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 1.0% 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 1.0% release of TCA-soluble counts.

#### **Double-Stranded 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 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:**

T3 DNA Ligase is supplied with 2X Rapid Ligation Buffer for use in ligation reactions.

The enzyme has also been characterized in the following buffer, with a 17-fold decrease in specific activity due to the absence of the crowding agent (PEG 6000):

1X T3 DNA Ligase Buffer:

50 mM Tris-HCl

300 mM NaCl

0.5 mM ATP

1 mM DTT

pH 8.0 @ 25°C

The following information was taken from the Cai reference (cited below), which describes the characterization of the T3 DNA Ligase:

Optimal pH: 8.0

Optimal reaction temperature: 20°C

Optimal reaction time: >30 minutes (without PEG 6000)

Optimal ionic strength: 300 mM NaCl

Optimal divalent cation/concentration: 2 mM  $Mg^{++}$

Optimal cofactor: ATP (0.5 mM)

### **References:**

1. Cai, Liang, et al. (2004) J. Biochem. 135, 397-403



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### **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.