



Product Information	
<b>T4 DNA Ligase</b>	
Part Number:	L603-HC-L
Lot Number:	

Rev. E

### **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**

B101 (2X Rapid Ligation Buffer)  
B603 (10X T4 DNA Ligase Buffer)

### **2X Rapid Ligation Buffer**

132mM Tris-HCl  
20 mM MgCl<sub>2</sub>  
2 mM dithiothreitol  
2 mM ATP  
15% PEG  
pH 7.6 @ 25°C

### **10X T4 DNA Ligase Buffer (B603)**

500mM Tris-HCl  
100 mM MgCl<sub>2</sub>  
50 mM dithiothreitol  
10 mM ATP  
pH 7.6 @ 25°C

### Product Specification\*

Unit Size	240,000 Units
Unit Concentration	600,000 U/mL
Volume	0.4 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	6,000 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]<sub>f</sub> = 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 µL reaction containing 15,000 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 10,000 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 pENZuC 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.

### **Real-Time PCR DNA 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:**

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.

T4 DNA Ligase is ATP dependent. It is recommended that the reaction buffer be discarded after one year of storage at -20°C and replaced with fresh buffer to ensure maximum performance.

Single-insert ligations are optimal when targeting an insert:vector ratio between 2 and 6. A ratio above 6:1 will promote the insertion of multiple fragments, while dropping below 2:1 will reduce ligation efficiency. For problematic ligations or if the DNA concentration is unknown, it may be necessary to vary ratios and run multiple ligations.

The presence of PEG at a high concentration will significantly reduce the transformation efficiency of electrocompetent cells. In order to maximize the efficiency of transformation into electrocompetent cells, the following approaches are recommended:

**Best:** Following ligation, purify the product using a DNA purification spin column and elute in 50 µL of TE. The DNA is now ready for transformation. The final amount of DNA to be transformed should be in the range of 0.1-10 ng.

**Better:** Dilute ligation product in ddH<sub>2</sub>O or TE to reduce the PEG concentration. The final amount of DNA to be transformed should be in the range of 0.1-10 ng.

Enzymatics' high-concentration T4 DNA Ligase in combination with the 2X Rapid Ligation buffer greatly stimulates the rate and efficiency blunt-end ligation, therefore long incubations (>10 minutes) are NOT recommended and can greatly reduce the transformation efficiency of ligation products. In order to maximize transformation efficiency of the correct insert/vector combination, the following protocol is recommended.

Enzymatics 10X T4 DNA Ligase Buffer does not contain PEG and is compatible with standard ligation protocols which do not specify the use of a rapid/fast/quick format buffer.

### **Usage Protocol (Rapid Ligation):**

Reaction Set-Up:

Amount	Description	Final Concentration
10 µL	2X Rapid Ligation Buffer	1X
X µL	Vector	1-10 ng/ µL
X µL	Insert	1-10 ng/ µL
1 µL	T4 DNA Ligase (600 U/ µL)	30 U/ µL
X µL	Type I Water	N/A
<b>20 µL</b>	<b>Total Volume</b>	

1. Add all of above components to a clean reaction vessel, mix well by pipetting.
2. Incubate at 25°C for 10 minutes.
3. Immediately purify DNA using PCR clean-up column and elute in ~50 µL.
4. - OR - Immediately dilute (at least 1:10, but enough such that 0.1-10 ng of ligation product will be transformed) in TE or water
5. Transform 0.1-10 ng of ligation product into chemically or electrocompetent cell line that is compatible with vector

### **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.