



Product Information	
<b>T4 RNA Ligase 1</b>	
Part Number:	L605L
Lot Number:	

Certificate of Analysis

2010-L605-Rev.E

### Product Description:

T4 RNA Ligase catalyzes the ATP-dependent ligation of single-stranded nucleic acids (RNA or DNA) (1,2).

### Source of Protein

A recombinant *E. coli* strain carrying the T4 RNA Ligase gene from bacteriophage T4.

### Supplied in

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

### Supplied With

B605 (10X T4 RNA Ligase Buffer)

### 10X T4 RNA Ligase Buffer (B605)

500 mM Tris-HCl  
100 mM MgCl<sub>2</sub>  
10 mM ATP  
100 mM Dithiothreitol  
pH 7.8 @ 25°C

### Unit Definition

One unit is defined as the amount of enzyme required to ligate 50% of 0.4 µg of an equimolar mix of two single stranded 23 base RNA oligonucleotides (one 5'-phosphorylated) in 20 µL 1X T4 RNA Ligase Buffer following a 30 minute incubation at 37°C.

### Product Specification\*

Unit Size:	10,000 Units
Unit Concentration	20,000 U/mL
Volume	0.5 mL
Purity (SDS-PAGE)	>99%
Specific Activity	16,800 U/mg
SS Exonuclease	200 U <5.0% released
DS Exonuclease	200 U <0.5% released
Endonuclease	200 U <10% converted
Non-specific RNase	200 U none detected
<i>E. coli</i> 16S rDNA Contamination	200 U <10 copies
Storage	-20°C

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

### Quality Control Analysis:

#### Unit Characterization Assay

Specific activity was measured using a 2-fold serial dilution method. Dilutions of enzyme were made in 1X T4 RNA Ligase reaction buffer ([T4 RNA Ligase]<sub>f</sub> = 1.0-0.008 µg/µL) and added to 20 µL reactions containing 0.4 µg of an equimolar mix of two single-stranded 23 base RNA oligonucleotides (one 5'-phosphorylated) and 1X T4 RNA Ligase Buffer. Reactions were incubated 30 minutes at 37°C, stopped, and analyzed on a 15% TBE-Urea gel stained with SYBR® Gold Nucleic Acid Gel Stain (Invitrogen S-11494).

#### 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 49,070 and molecular weight of 43,509 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.

## **Nuclease Contamination Tests:**

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

A 50 µl reaction containing 11,000 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 5.0% release of TCA-soluble counts.

### **Double-Stranded Exonuclease Activity**

A 50 µl reaction containing 5,000 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 0.5% release of TCA-soluble counts.

### **Endonuclease Activity**

A 50 µL reaction containing 0.5 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.

### **Non-Specific RNase Assay**

Product was screened for non-specific RNase contamination using the RNase Alert kit, (Integrated DNA Technologies), following the manufacturer's guidelines.

### ***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.



#### **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.