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# Product Information Sheet

| Product Information | |
|---|---|
| **T4 RNA Ligase 1** | |
| **Part Number** | L6050L 280230 |
| | |
| **Concentration** | 20,000 U/mL |
| **Unit Size** | 10,000 U |

| Product Specification | |
|---|---|
| **Storage Temperature** | -25°C to -15°C |
| **TEST:** | **SPECIFICATION:** |
| **Purity (SDS-PAGE)** | >99% |
| **Specific Activity** | 16,800 U/mg |
| **SS Exonuclease** | 200 U <5.0% released |
| **DS Exonuclease** | 200 U <1.0% released |
| **DS Endonuclease** | 200 U =No conversion |
| ***E.coli* DNA Contamination** | 200 U <10 copies |
| **Non-specific RNAse** | 200 U none detected |

## 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 DTT
50% glycerol
pH 7.5 @ 25°C

### Supplied with

B6050 (10X T4 RNA Ligase Buffer)

**10X T4 RNA Ligase Buffer (B6050):**
500 mM Tris-HCl
100 mM $MgCl_2$
10 mM ATP
100 mM DTT
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.

## 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 ($[T4\ RNA\ Ligase]_f$ = 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 ($OD_{280}$) Measurement

A 2.0 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 49,070 and molecular weight of 43,509 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 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 37ºC resulted in less than 1.0% release of TCA-soluble counts.

### Double-Stranded 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.

### *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.

### 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.

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Biozym Scientific GmbH
Steinbrinksweg 27
D-31840 Hessisch Oldendorf
Tel.: +49 (0)5152-9020
Fax: +49 (0)5152-2070
support@biozym.com
www.biozym.com

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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.