Product Information	
T4 RNA Ligase 2	
Part Number	L6080L
Concentration	30,000 U/mL
Unit Size	4,500 U

Product Specification	
Storage Temperature	-25°C to -15°C
TEST	**SPECIFICATION**
Purity (SDS-PAGE)	> 99%
Specific Activity	> 120,000 U/mg
SS Exonuclease	500 U < 5% Released
DS Exonuclease	500 U < 1% Released
DS Endonuclease	500 U = No Conversion
***E.coli* DNA Contamination**	500 U < 10 copies
RNAse Contamination	500 U = No detectable non-specific RNAse

Product Description:

T4 RNA Ligase 2 catalyzes phosphodiester bond formation between a 5' phosphate and 3' hydroxyl of RNA. The preferred substrate is nicked double-stranded RNA but single-stranded RNA can also serve as a substrate. Ligation of single-stranded RNA substrates generates either intramolecular or intermolecular products. Besides nicked double-stranded RNA substrates, other nicked nucleic acids hybrids can be sealed. The strand containing the 5' phosphate can either be DNA or RNA. The non-ligated strand of the duplex can be either RNA or DNA. T4 RNA ligase 2 requires ATP for activity unless the substrate is preadenylated on the 5' end. A truncated version of T4 RNA ligase 2 is a better enzyme for preadenylated substrates because it generates less side-reaction ligation products than the full length enzyme.

Source of Protein

Purified from a strain of *E. coli* that expresses the recombinant T4 RNA Ligase 2 gene.

Supplied in

10 mM Tris-HCl
100 mM NaCl
0.1 mM DTT
0.1 mM EDTA
50% glycerol
pH 7.5 @ 25°C

Supplied with

B6030 10X Ligation Buffer

10X Ligation Buffer (B6030)

500 mM Tris-HCl
100 mM $MgCl_2$
50 mM DTT
10 mM ATP
pH 7.6 @ 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 a single-stranded 5' FAM-labeled 17-mer RNA to the 5' phosphorylated end of a 18-mer DNA when both strands are annealed to a complementary 35-mer DNA strand in 20 µL at 37°C for 30 minutes.

Quality Control Analysis:

Unit Characterization Assay

Specific activity was measured using a 2-fold serial dilution method. Dilutions of enzyme were made in 1X reaction buffer and 2 µL of each enzyme dilution was added to 18 µL reactions in 1X reaction buffer containing 0.4 µg of an equimolar mix of one 17 base RNA oligonucleotide (5' FAM-labeled) and one 18 base DNA oligonucleotide (5' phosphorylated) annealed to a complementary 35-mer DNA oligonucleotide. Reactions were incubated 30 minutes at 37°C, quenched, and analyzed on a 15% TBE-Urea gel.

Protein Concentration (OD_{280}) Measurement

A 2.0 µL sample of enzyme was analyzed at OD_{280} using a Nanodrop ND-2000 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 40,680 and molecular weight of 37,627 Daltons.

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.

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 37°C 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

Replicate 10 µL samples were screened for non-specific RNAse contamination using the RNAse Alert kit, (Integrated DNA Technologies), following the manufacturer's guidelines.

References:

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3. Nandakumar, J. et al. (2004) *J. Biol. Chem*, 279, 31337-31347.
4. Aravin, A. and Tusch, T. (2005) *FEBS Letters*, 579, 5830-5840.
5. Pfeffer, S. et al. (2005) *Nat. Meth*, 2, 269-276.

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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.