Product Information	
T4 RNA Ligase 2	
Part Number	L6080L
Concentration	30,000 U/mL
Unit Size	4,500 U
Storage Temperature	-25°C to -15°C
Lot Number	
Reference Number	

Product Description: T4 RNA Ligase 2 catalyzes phosphodiester bond formation between a 5' phosphate and 3' hydroxyl termini of RNA. The preferred substrate is nicked double-stranded RNA, but other nicked nucleic acid hybrids can also be sealed. T4 RNA ligase 2 requires ATP for activity unless the substrate is pre-adenylated on the 5' end (1-5).

Product Specifications							
L6080							
Assay	**SDS Purity**	**Specific Activity**	**SS Exonuclease**	**DS Exonuclease**	**DS Endonuclease**	***E. coli* DNA Contamination**	**Non-specific RNAse**
Units Tested	**n/a**	**n/a**	**500**	**500**	**500**	**500**	**500**
Specification	>99%	>120,000 U/mg	<5.0% Released	<1.0% Released	No Conversion	<10 copies	No detectable non-specific RNAse

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

Unit Definition: 1 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 an 18-mer DNA when both strands are annealed to a complementary 35-mer DNA strand in 20 µL at 37°C for 30 minutes.

Molecular weight: 37.6 kDa

Quality Control Analysis:

Unit Activity is 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}) is determined by OD_{280} absorbance.

Physical Purity is evaluated by SDS-PAGE of concentrated and diluted enzyme solutions followed by silver stain detection. Purity is assessed by comparing the aggregate mass of contaminant bands in the concentrated sample to the mass of the protein of interest band in the diluted sample.

Single-Stranded Exonuclease is determined in a 50 µL reaction containing a radiolabeled single-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.

Double-Stranded Exonuclease is determined in a 50 µL reaction containing a radiolabeled double-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.

Double-Stranded Endonuclease is determined in a 50 µL reaction containing 0.5 µg of plasmid DNA and 10 µL of enzyme solution incubated for 4 hours at 37°C.

***E. coli* 16S rDNA Contamination** is evaluated using 5 µL replicate samples of enzyme solution 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.

Non-Specific RNAse contamination is assessed using the RNAse Alert kit, (Integrated DNA Technologies), following the manufacturer's guidelines.

Supplied in:
10 mM Tris-HCl, 100 mM NaCl, 0.1 mM DTT, 0.1 mM EDTA, 50% glycerol (pH 7.5 at 25°C)

Supplied with:
10X Ligation Buffer (B6030): 500 mM Tris-HCl, 100 mM $MgCl_2$, 50 mM DTT, 10 mM ATP (pH 7.6 at 25°C)

Usage Instructions: Nick ligation in double-stranded RNA

1. Set up the following reaction mixture in a total volume of 20 µL:

Components	Final Concentration	Volume
Nuclease free water	N/A	X µL
10X T4 RNA Ligase Buffer (B6030)	1X	2 µL
Nicked dsRNA substrate	1 µM	X µL
T4 RNA Ligase 2 (L6080L)	10 U	0.33 µL
	Total Volume =	20 µL

2. Incubate at 25°C for 60 minutes.
3. Reaction can be stopped by adding EDTA and incubation at 65°C for 20 min, or clean-up by using a spin column-based method.

References:
1. Ho, C.K. et al. (2004) Structure, 12, 327-339.
2. Ho, C.K. and Shuman, S. (2002) Proc. Natl. Acad. Sci. USA, 99, 12709-12714.
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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