Product Information	
T4 RNA Ligase 2, Truncated	
Part Number	L6070L
Concentration	5,000 U/mL
Unit Size	500 U
Storage Temperature	-25°C to -15°C
Lot Number	
Reference Number	

Product Description: T4 RNA Ligase 2 truncated catalyzes phosphodiester bond formation between a pre-adenylated 5' phosphate (DNA or RNA) and the 3' hydroxyl of RNA. The truncated enzyme contains the first 249 amino acids which makes the enzyme require a pre-adenylated 5' terminal donor and eliminates the need for ATP. Because T4 RNA ligase 2 truncated cannot use the 5' phosphate of RNA or DNA as a donor in the ligation reaction, it is useful for certain applications such as linker ligations with pre-adenylated 5' DNA to 3' hydroxyl RNA. The desired specific ligation products are enhanced dramatically over unwanted background ligation products, making the truncated enzyme superior to the full-length enzyme for this use (1-5).

Product Specifications							
L6070							
Assay	**SDS Purity**	**Specific Activity**	**SS Exonuclease**	**DS Exonuclease**	**DS Endonuclease**	***E. coli* DNA Contamination**	**Non-specific RNase**
Units Tested	**n/a**	**n/a**	**50**	**50**	**50**	**50**	**50**
Specification	>99%	30,000 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 truncated 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' pre-adenylated end of an 18-mer DNA when both 17-mers are annealed to a complementary 35-mer DNA strand in 20 µL 1X reaction buffer following 30 minutes of incubation at 37°C.

Molecular weight: 30.5 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' pre-adenylated) 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 T4 RNA Ligase 2, Truncated Reaction Buffer (B6070): 500 mM Tris-HCl, 100 mM $MgCl_2$, 50 mM DTT (pH 7.6 at 25°C)

Usage Instructions: Ligation of the 3' OH of RNA to the 5' pre-adenylated DNA

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 2, Truncated Reaction Buffer (B6070)	1X	2 µL
3' OH RNA	1 µM	X µL
5' pre-adenylated DNA or RNA	2 µM	X µL
T4 RNA Ligase 2, Truncated (L6070L)	5 U	1 µL
	Total Volume =	20 µL

2. Incubate at 37°C for 30 minutes.
3. Reaction can be stopped by adding EDTA or incubation at 75°C for 10 minutes.

Notes:
Enzymatics' T4 RNA Ligase 2 Truncated demonstrates equivalent or superior volume/volume performance when compared to analogous T4 RNA Ligase 2 Truncated products on the market.

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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