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Product Information Sheet

Product Information	
T4 RNA Ligase 2, Truncated	
Part Number	L6070L 280245
Price	
Concentration	5,000 U/mL
Unit Size	500 U

Product Specification	
Storage Temperature	-25°C to -15°C
TEST	**SPECIFICATION**
Purity (SDS-PAGE)	> 99%
Specific Activity	30,000 U/mg
SS Exonuclease	50 U < 5% Released
DS Exonuclease	50 U < 1% Released
DS Endonuclease	50 U = No Conversion
***E.coli* DNA Contamination**	50 U < 10 copies
RNAse Contamination	50 U = No detectable non-specific RNAse

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.

Source of Protein

Purified from a strain of *E. coli* that expresses the recombinant truncated 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

B6070 10X T4 RNA Ligase 2, Truncated Buffer

10X T4 RNA Ligase 2, Truncated Buffer (B6070)

500 mM Tris-HCl
100 mM $MgCl_2$
50 mM DTT
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' pre-adenylated end of a 18-mer DNA when both 17-mers are annealed to a complementary 35-mer DNA strand in 20 µL 1X reaction 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 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}) Measurement

The enzyme was analyzed at OD_{280} using a Nanodrop spectrophotometer standardized with a 2.0 mg/ml BSA sample and blanked with product storage solution. The observed average measurement of 3 replicate samples was converted to mg/mL using an extinction coefficient of 33,710 and molecular weight of 30,451 Daltons.

SDS-Page (Physical Purity Assessment)

A concentrated sample of enzyme was loaded on a denaturing 4-20% Tris-Glycine SDS-PAGE gel flanked by a broad-range MW marker and 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. Comparison between the concentrated and the diluted samples is used to evaluate percent purity.

Contamination Tests:

Single-Stranded Exonuclease Activity

Radiolabeled single-stranded DNA substrate incubated with enzyme solution for 4 hours at 37°C resulted in less than 5% release of TCA-soluble counts.

Double-Stranded Exonuclease Activity

Radiolabeled double-stranded DNA substrate incubated with enzyme solution for 4 hours at 37°C resulted in less than 1% release of TCA-soluble counts.

Double-Stranded Endonuclease Activity

A reaction containing plasmid DNA incubated with enzyme solution 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 samples of enzyme 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

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

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:

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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.