

Product Information						
RNAse Inhibitor						
Part Number	Y9240L					
Concentration	40,000 U/mL					
Unit Size	20,000 U					
Storage Temperature	-25ºC to -15ºC					
Lot Number						
Reference Number						

Product Specifications Y9240L Rev 02

Product Description: RNAse Inhibitor is an acidic, 52 kDa protein that is a potent non-competitive inhibitor of pancreatic-type ribonucleases such as RNase A, RNase B, and RNase C. The enzyme is provided as a fusion of the porcine RNAse Inhibitor gene with a proprietary, 22.5 kDa protein tag.

Product Specifications									
Assay	SDS	Specific	SS	DS	DS	E. coli DNA	Non-specific		
	Purity	Activity	Exonuclease	Exonuclease	Endonuclease	Contamination	RNAse		
Units Tested	n/a	n/a	2,000	2,000	2,000	2,000	2,000		
Specification	>99%	53,333U/mg	<5.0%	<1.0%	No Conversion	<10 copies	No detectable non-		
			Released	Released			specific RNAse		

Source of Protein: A recombinant E. coli strain carrying the porcine RNAse Inhibitor gene.

<u>Unit Definition:</u> 1 unit is defined as the amount of enzyme required to inhibit by 50% the hydrolysis of cytidine 2',3'-cyclic monophosphate by 5 ng of RNAse A (1).

Molecular weight: 74,828 Daltons

Quality Control Analysis:

Unit Activity is determined using 2-fold serialserial dilution method. Dilutions of enzyme were made in 1X RNAse Inhibitor Reaction Buffer and added to 1000 µL reactions containing 1mM cytidine 2',3'-cyclic monophosphate, 1µg RNase A in a 1X reaction buffer containing 100mM Tris-Acetate, 1mM EDTA, pH 6.5. Absorbance at 286nm was observed at 20 second intervals during a 5minute reaction.

Protein Concentration (OD280) is determined by OD280 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 μ L reaction containing a radiolabeled single-stranded DNA substrate and 10 μ L of enzyme solution incubated for 4 hours at 37°C.

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

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

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. SDS sheets relevant to this product are available upon request. 100 Cummings Center, Suite 407J, Beverly, MA 01915 • Ph (888) 927-7027 • Fax (978) 867-5724 • <u>www.enzymatics.com</u> FMWI016.1 Rev 01



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: 20mM Hepes-KOH, 50mM KCl, 8mM DTT, 50% glycerol (pH 7.5 at 25°C).

<u>Usage Instructions</u>: Use of RNase Inhibitor in cDNA synthesis, in vitro transcription or in vitro translation reactions to preserve RNA integrity and prevent degradation by RNase contamination.

Add RNase inhibitor (final concentration of 1 U/ μ L) to the reaction mixture before other potentially RNase contaminated components (e.g. enzyme, BSA, template nucleic acids) are added.

RNAse Inhibitor is an acidic, 52 kDa protein that is a potent non-competitive inhibitor of pancreatic-type ribonucleases such as RNase A, RNase B, and RNase C and is active below 50°C and under non-denaturing conditions.

References:

1. Blackburn, P., 1979. Ribonulcease Inhibitor from Human Placenta: Rapid Purification and Assay. The Journal of Biological Chemistry, Vol. 254, No. 24 pp 12484-12487.

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