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Product Information Sheet *Y9240L Rev D*

Product Information	
RNAse Inhibitor	
Part Number	Y9240L
Concentration	40,000 U/mL
Unit Size	20,000 U

Product Specification	
Storage Temperature	-25°C to -15°C
TEST:	**SPECIFICATION:**
Purity (SDS-PAGE)	>99%
SS Exonuclease	2000 U <5.0% released
DS Exonuclease	2000 U <1.0% released
DS Endonuclease	2000 U =No conversion
***E.coli* DNA Contamination**	2000 U <10 copies
RNAse Contamination	2,000 U = No Detectable non-specific RNase

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.

Source of Protein

A recombinant *E. coli* strain carrying the porcine RNAse Inhibitor gene.

Supplied in

20 mM Hepes-KOH
50 mM KCl
8 mM DTT
50% glycerol
pH 7.5 @ 25°C

Unit Definition

One 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)

Quality Control Analysis:

Unit Characterization Assay

Specific activity was determined using 1.1-fold serial 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 (OD_{280}) Measurement

A 2.0 µL sample of enzyme was analyzed at OD_{280} using a Nanodrop ND-1000 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 59,600 and molecular weight of 74,828 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

(additional information on reverse side)

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 37ºC 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.

Non-Specific RNAse Assay

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

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.

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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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.