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Product Information Sheet *Y9220L Rev D*

Product Information	
RNAse H	
Part Number	Y9220L 280510
Concentration	5,000 U/mL
Unit Size	5,000 U

Product Specification	
Storage Temperature	-25°C to -15°C
TEST:	**SPECIFICATION:**
Purity (SDS-PAGE)	>99%
Specific Activity	625,000 U/mg
SS Exonuclease	500 U <5.0% released
DS Exonuclease	500 U <1.0% released
DS Endonuclease	500 U =No conversion
***E.coli* DNA Contamination**	500 U <10 copies
RNAse Contamination	500 U = No Detectable non-specific RNase

Product Description:

E.coli RNase H (rnh) is an endoribonuclease which degrades the RNA strand of RNA/DNA hybrid molecules. RNase H digestion produces ribonucleotide molecules with 5-phosphate and 3-hydroxyl termini. RNAse H is nearly inactive against single or double-stranded RNA molecules.

Source of Protein

A recombinant *E. coli* strain carrying the RNAse H (rnh) gene from *E.coli*.

Supplied in

20 mM Tris-HCl
100 mM KCl
10 mM $MgCl_2$
0.1 mM EDTA
0.1 mM DTT
50% glycerol
pH 7.9 @ 25°C

Supplied with

B9220 (10X RNAse H Buffer)

10X RNAse H Buffer (B9220):

500 mM Tris-HCl
750 mM KCl
30 mM $MgCl_2$
100 mM DTT
pH 8.3 @ 25°C

Unit Definition

1 unit is defined as the amount of enzyme that will hydrolyze 1 nmol of RNA from an ^{3}H-labeled DNA:RNA hybrid molecule into acid-soluble material in 20 minutes 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 RNAse H reaction buffer and added to 50 µL reactions containing ^{3}H-labeled poly(rA), poly (dT) DNA, and 1X RNAse H Buffer. Reactions were incubated 20 minutes at 37°C, plunged on ice, and release of TCA soluble counts was analyzed.

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 40,540 and molecular weight of 18,053 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 incubated for 4 hours at 37°C resulted in less than 5.0% release of TCA-soluble counts.

(additional information on reverse side)

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.

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.

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.

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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.