



Product Information

RNAse H

Part Number:	Y922L
Lot Number:	

Certificate of Analysis

2010-Y922-Rev.C

For Research Use Only

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 MgCl₂
0.1 mM EDTA
0.1 mM dithiothreitol
50% glycerol
pH 7.9 @ 25°C

Supplied With

B922 (10X RNAse H Buffer)

10X RNAse H Buffer (B922)

500 mM Tris-HCl
750 mM KCl
30 mM MgCl₂
100 mM dithiothreitol
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 ³H-labeled DNA:RNA hybrid molecule into acid-soluble material in 20 minutes at 37°C.

Product Specification*

Unit Size:	5,000 Units
Unit Concentration	5,000 U/mL
Volume	1.0 mL
Purity (SDS-PAGE)	>99%
Specific Activity	625,000 U/mg
SS Exonuclease	500 U <5.0% released
DS Exonuclease	500 U <0.5% released
Endonuclease	500 U <10% converted
Non-specific RNAse	500 U none detected
<i>E. coli</i> 16S rDNA Contamination	500 U <10 copies
Storage	-20°C

* For a detailed summary of assay conditions and data, refer to the Quality Controls Analysis section below

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 ([RNAse H]_f = 0.16-0.02 µg/mL) and added to 50 µL reactions containing 10 nmol ³H-labeled poly(rA) and 12.5 µg poly (dT), 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 (OD₂₈₀) Measurement

A 2.0 µL sample of enzyme was analyzed at OD₂₈₀ 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 17,600 Daltons. Acceptance for this assay is +/- 5% of reference sample.

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.

(additional information on reverse side)

Nuclease Contamination Tests:

Single-Stranded Exonuclease Activity

A 50 µl reaction containing 11,000 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.

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 37°C resulted in less than 0.5% release of TCA-soluble counts.

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.



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.