Product Information	
RNAse H	
Part Number	Y9220L
Concentration	5,000 U/mL
Unit Size	5,000 U
Storage Temperature	-25°C to -15°C
Lot Number	
Reference Number	

Product Description: *E. coli* RNAse H (rnh) is an endoribonuclease which degrades the RNA strand of RNA/DNA hybrid molecules (1,2). 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.

Product Specifications							
Y9220							
Assay	**SDS Purity**	**Specific Activity**	**SS Exonuclease**	**DS Exonuclease**	**DS Endonuclease**	***E. coli* DNA Contamination**	**Non-specific RNAse**
Units Tested	**n/a**	**n/a**	**500**	**500**	**500**	**500**	**500**
Specification	>99%	625,000 U/mg	<5.0% Released	<1.0% Released	No Conversion	<10 copies	No detectable non-specific RNAse

Source of Protein: Purified from a recombinant *E. coli* strain carrying the RNAse H (*rnh*) gene from *E. coli*.

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.

Molecular weight: 18.1 kDa

Quality Control Analysis:

Unit Activity is 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}) is determined by OD_{280} 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 µL reaction containing a radiolabeled single-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.

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

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

***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:
20 mM Tris-HCl, 100 mM KCl, 0.1 mM DTT, 10 mM $MgCl_2$, 0.1 mM EDTA, 50% glycerol (pH 7.9 at 25°C)

Supplied with:
10X RNAse H Buffer (B9220): 500 mM Tris-HCl, 750 mM KCl, 30 mM $MgCl_2$, 100 mM DTT (pH 8.3 at 25°C)

Usage Instructions:
1. Set up the following reaction mixture in a total volume of 100 µL:

Components	Final Concentration	Volume
Nuclease free water	N/A	X µL
10X RNAse H Buffer (B9220)	1X	10 µL
RNA: DNA duplex	2 µg	X µL
RNAse H (Y9220L)	5 U	1 µL
	Total Volume =	100 µL

2. Incubate the reaction at 37°C for 20 minutes.
3. Stop the reaction by adding 1 µL of 0.5 M EDTA.

References:
1. Donnis-Keller, H. (1979) Nucl. Acids Res., 7, 179.
2. Schultz, S.J. and Champoux, J.J. (2008) Virus Res., 134(1-2): 86-103.

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