Ribonuclease H, *E. coli* (Cloned)

Store at –20°C
Do not store in a frost-free freezer

Catalog # (P/N):	AM2292	AM2293
Amount:	200 Units	1,000 Units

Product Description: A high purity Ribonuclease H, suitable for most RNA amplification and nucleic acid sequence-based amplification (NASBA) protocols.

Source: An *E. coli* strain overexpressing RNase H.

Concentration: 10 U/µL

Unit Definition: One unit of Ribonuclease H is the amount of enzyme required to increase fluorescence 1.5 RFUs per sec at 37°C using 20 pmol of RNaseAlert® probe coupled to 1000 pmol of a complementary oligonucleotide as substrate.

Materials Not Provided: Because of the variety of product applications, no reaction buffer is supplied.

Storage Conditions: Store at –20°C. ***Do not store in a frost-free freezer.***

Storage Buffer: (Not included) 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1% TritonX-100, and 50% glycerol.

USER INFORMATION

General Information: Ribonuclease H (RNase H) specifically degrades the RNA in RNA:DNA hybrids to produce 3'−hydroxyl and 5'−phosphate terminated products. The enzyme will not degrade single-stranded DNA, double-stranded DNA, or unhybridized RNA. RNase H is an essential component of the Ambion MessageAmp™ II aRNA Amplification Kit (P/N AM1751), which is based on the patented Eberwine aRNA amplification procedure. RNase H can also be used to degrade a specific RNA when the complementary DNA oligonucleotide is hybridized to it, as is used in antisense technology, and for poly(A) tail removal from mRNA hybridized to oligo(dT).

Applications: The following general protocol for digestion of RNA;DNA hybrids is adapted from *Current Protocols in Molecular Biology*. The general reaction parameters, including the amount of enzyme, and the method of stopping the reaction, will vary depending on your experimental needs.

Digestion of RNA:DNA hybrids
20 mM HEPES-KOH, pH 8.0
50 mM KCl
4 mM $MgCl_2$
1 mM DTT
2 µg RNA:DNA hybrid
50 µg/mL BSA
~10 unit Ribonuclease H per µg RNA:DNA hybrid
Total reaction volume: 100 µL

Incubate 20 min at 37°C
Add 1 µL 0.5 M EDTA to stop the reaction.

Reference: Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K, editors. (2006) Enzymatic Manipulation of DNA and RNA (Chapter 3). In *Current Protocols in Molecular Biology*. John Wiley & Sons, Inc. p 3.13.2.

QUALITY CONTROL

Ribonuclease H is rigorously tested for contaminating nonspecific endonuclease, exonuclease, RNase, and protease activity.

OTHER INFORMATION

Material Safety Data Sheets: Material Safety Data Sheets (MSDSs) can be printed or downloaded from product-specific links on our website at the following address: www.ambion.com/techlib/msds. Alternatively, e-mail your request to MSDS_Inquiry_CCRM@appliedbiosystems.com. Specify the catalog or part number(s) of the product(s), and we will e-mail the associated MSDSs unless you specify a preference for fax delivery. For customers without access to the internet or fax, our technical service department can fulfill MSDS requests placed by telephone or postal mail. (Requests for postal delivery require 1–2 weeks for processing.)

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