RNase H

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M0297S 005141016101

M0297S

RR 37° Yes

250 units **5,000 U/ml** **Lot: 0051410**

RECOMBINANT **Store at –20°C** **Exp: 10/16**

Description: Ribonuclease H (RNase) H is an endoribonuclease which specifically hydrolyzes the phosphodiester bonds of RNA which is hybridized to DNA. This enzyme does not digest single or double-stranded DNA.

Source: An *E. coli* strain that carries the cloned RNase H gene (rnh) from *Escherichia coli*

Applications:

- Removal of poly(A) tails of mRNA hybridized to poly(dT)
- Removal of mRNA during second strand cDNA synthesis

Supplied in: 100 mM KCl, 20 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 0.1 mM EDTA, 0.1 mM dithiothreitol and 50% glycerol.

Reagents Supplied with Enzyme:
10X RNase H Reaction Buffer.

Reaction Conditions: 1X RNase H Reaction Buffer. Incubate at 37°C.

1X RNase H Reaction Buffer:
75 mM KCl
50 mM Tris-HCl
3 mM $MgCl_2$
10 mM dithiothreitol
pH 8.3 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme that will hydrolyze 1 nmol of the RNA in [^{3}H]-labeled poly(rA)•poly(dT), to acid-soluble ribonucleotides in a total reaction volume of 50 µl in 20 minutes at 37°C.

Unit Assay Conditions: 1X RNase H Reaction Buffer, 10 nmol [^{3}H]-labeled poly(rA) and 12.5 µg poly(dT).

Quality Control Assays

RNase Assay: Incubation of a 10 µl reaction containing 50 units of RNase H with 40 ng of RNA transcripts for 1 hour at 37°C results in no detectable degradation of the RNA as determined by gel electrophoresis.

SS DNA Exonuclease Activity: Incubation of 50 units of enzyme with 1 µg of sonicated and denatured [^{3}H]-DNA (10^5 cpm/ug) for 30 minutes at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

Endonuclease Activity: Incubation of a 50 µl reaction containing 50 units of RNase H with 1 µg of ϕX174 RF I DNA for 4 hours at 37°C resulted in < 10% conversion to RFII as determined by agarose gel electrophoresis.

Heat Inactivation: 65°C for 20 minutes.

References:

1. Gubbler, U. and Hoffman, B.J. (1983) *Gene* 25, 263–269.
2. Davis, R. et al. (1988) *Cell Biol.* 8, 4745–4755.
3. Donnis-Keller, H. (1979) *Nucleic Acid Res.* 7, 179.
4. Goodwin, E. C. and Rottman, F.M. (1992) *Nucleic Acids Res.* 20, 916.

CERTIFICATE OF ANALYSIS

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