Ribonuclease H (RNase H)

From Escherichia coli H 560 pol A1

Cat. No. 10 786 357 001 100 U

1. Product Overview

Volume Activity
Approximately 1 U/µl

Unit Definition
RNase H is assayed according to Hillenbrand and Staudenbauer (1). One unit of RNase H is the amount of enzyme which produces 1 nmol acid-soluble ribonucleotides from [3H] poly(A) × poly(dT) in 20 min at +37°C under the stated assay conditions.

Specific Activity
Approximately 40,000 U/mg according to (1). Protein determination according to a modified Lowry procedure (2, 3).

Storage Buffer
25 mM Tris-HCl, 50 mM KCl, 1 mM dithiothreitol, 0.1 mM EDTA, 50% glycerol (v/v), pH approximately 8.0.

Definition
By convention, the enzyme activity in E. coli against the RNA of RNA/DNA hybrids is designated as “RNase H”, whereas the activity against the RNA in RNA/RNA duplexes is named “RNase III” (4).

Description
The endoribonuclease RNase H degrades the RNA strand of RNA/DNA hybrids of natural origin, e.g., that of phage φX174, and of synthetic complexes, e.g., poly(A) × poly(dT). RNase H produces ribonucleotides with 5´-phosphate and 3´-OH termini. Nearly no activity is detected with polyribonucleotides alone or polymers annealed to their complementary ribopolymer (5, 6).

Properties
• RNase H requires Mg²⁺ for optimal activity; Mg²⁺ can be only partially replaced by Mn²⁺. The enzyme has its maximal activity in the presence of SH-reagents and is inhibited by N-ethylmaleimide.
• The optimum pH is 7.5 to 9.1 (5).
• RNase H activity is relatively insensitive to salt; 50% of its activity is retained in the presence of 0.3 M NaCl (5).
• RNase H hydrolyzes poly(A) × poly(dT) and RNA/DNA hybrids of φX174 at equal rates.
• In the presence of dextran, the degradation of poly(A) × poly(dT) is inhibited, while that of RNA/DNA hybrids of φX174 is not (6). Other saccharides fail to inhibit RNase H (6).

Application
In addition to the use of E. coli RNase H to study the in vivo RNA-primed initiations of DNA synthesis (1), RNase H is applied in the synthesis of cDNA. This is achieved by combining classical first-strand synthesis with the novel DNA polymerase I, RNase H and E. coli DNA ligase-mediated second-strand synthesis (7, 8). Additionally, RNase H is used to detect RNA/DNA regions in dsDNA of natural origin (9, 10). A further application is the removal of poly(A) sequences of mRNA, which leads to increased electrophoretic homogeneity of mRNA in gel electrophoresis (11).

RNase H may also be used for the site-specific enzymatic cleavage of RNA. With this method, a synthetic DNA oligomer will hybridize only to complementary single-stranded regions of an RNA molecule which therefore are digested by RNase H in a site-specific manner (12).

Storage and Stability
Store the unopened reagent at −15 to −25°C until the expiration date printed on the label.

2. Assay System

Incubation Buffer
20 mM HEPES-KOH, 50 mM KCl, 10 mM MgCl₂, 1 mM dithiothreitol, pH 8.0 (at +37°C).

Substrate Solution
200 µg poly(A) and 200 µg poly(dT) are dissolved in a total volume of 1,000 µl incubation buffer. The mixture is heated for 5 min at +95°C and then allowed to cool slowly for 2 hours to generate a RNA/DNA hybrid.

Assay Procedure
After a 10 min preincubation at +37°C 125 µl of substrate solution (including 50 µg of RNA/DNA Hybrid) are mixed with various amounts of RNase H (15 to 50 U) and incubation buffer in a total volume of 1,000 µl. The volume activity is determined by measuring the increase of absorbance at 264 nm.

3. Quality Control

Absence of Endonuclease
1 µg λ DNA is incubated with RNase H for 1 hour at +37°C in 25 µl of incubation buffer. Incubation with up to 10 U RNase H does not degrade λ DNA.

Absence of Nicking Activities
1 µg supercoiled pBR322 DNA is incubated with RNase H for 1 hour at +37°C in 25 µl incubation buffer. Incubation with up to 10 U RNase H does not show relaxation of supercoiled pBR322 DNA.

Absence of Ribonucleases
5 µg MS2 RNA are incubated with RNase H for 1 hour at +37°C in 50 µl incubation buffer. Incubation with up to 10 U RNase H does not show degradation of MS2 RNA.

Changes to Previous Version
• Editorial changes

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References
2 Lowry, O. H. et al. (1951) J. Biol. Chem. 183, 265.
5 Berkower, I. et al. (1973) J. Biol. Chem. 248, 5914.

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