For life science research only.
Not for use in diagnostic procedures.

Ribonuclease H (RNase H)
from *Escherichia coli* H 560 *pol* A1

Version: 19
Content Version: November 2021

Cat. No. 10 786 357 001 100 U
1 U/µl

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	Ribonuclease H (RNase H)	Storage buffer: 25 mM Tris-HCl, 50 mM KCl, 1 mM dithiothreitol, 0.1 mM EDTA, 50% glycerol (v/v), pH approximately 8.0.	1 vial, 100 U

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the product is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	Ribonuclease H (RNase H)	Store at −15 to −25°C.

1.3. Additional Equipment and Reagent required

For preparation of solutions

- 20 mM HEPES-KOH
- 50 mM KCl
- 10 mM $MgCl_2$
- DTT*
- Poly(A)
- Poly(dT)

1.4. Application

In addition to the use of *E. coli* RNase H to study the *in vivo* RNA-primed initiations of DNA synthesis, 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. The enzyme can also be used for the:

- Detection of RNA/DNA regions in dsDNA of natural origin.
- Removal of poly(A) sequences of mRNA, which leads to increased electrophoretic homogeneity of mRNA in gel electrophoresis.
- 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.

2. How to Use this Product

2.1. Before you Begin

Mg^{2+} Concentration

RNase H requires Mg^{2+} for optimal activity. Mg^{2+} can be only partially replaced by Mn^{2+}.

General Considerations

RNase H hydrolyzes poly(A) × poly(dT) and RNA/DNA hybrids of ΦX174 at equal rates.

Working Solution

Incubation buffer

20 mM HEPES-KOH, 50 mM KCl, 10 mM $MgCl_2$, 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 minutes at +95°C and then allowed to cool slowly for 2 hours to generate a RNA/DNA hybrid.

2.2. Protocols

Standard assay

See section, **Working Solution** *for information on preparing solutions.*
After 10 minutes 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 absorbency at 264 nm.

2.3. Parameters

Activator

The enzyme has its maximal activity in the presence of SH-reagents and is inhibited by N-ethylmaleimide.

Inhibition

In the presence of dextran, the degradation of poly(A) × poly(dT) is inhibited, while that of RNA:DNA hybrids of ΦX174 is not. Other saccharides fail to inhibit RNase H.

pH Optimum

7.5 to 9.1

Sensitivity

RNase H activity is relatively insensitive to salt. 50% of its activity is retained in the presence of 0.3 M NaCl.

Specific Activity

Approximately 40,000 U/mg.

Unit Definition

One unit of RNase H is the amount of enzyme which produces 1 nmol acid-soluble ribonucleotides from [^{3}H] poly(A) × poly(dT) in 20 minutes at +37°C under the stated assay conditions.

Volume Activity

Approximately 1 U/µl

3. Additional Information on this Product

3.1. Test Principle

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.

- The endoribonuclease RNase H degrades the RNA strand of RNA/DNA hybrids of natural origin, such as that of phage ΦX174, and of synthetic complexes, such as 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.

3.2. Quality Control

For lot-specific certificates of analysis, see section, **Contact and Support**.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
1,4-Dithiothreitol	2 g	10 197 777 001
	10 g	10 708 984 001
	25 g	11 583 786 001

4.4. Trademarks

All product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products and select the corresponding product catalog.

4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed

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