Thermo SCIENTIFIC

PRODUCT INFORMATION

RNase H

#_ **_**

Lot: _ **Expiry Date: _**

Concentration: 5 u/µl

Supplied with: 1 ml of 10X Reaction Buffer

Store at -20°C

BSA included

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Description

Ribonuclease H (RNase H) specifically degrades the RNA strand in RNA-DNA hybrids. It does not hydrolyze the phosphodiester bonds within single-stranded and double-stranded DNA and RNA.

Applications

- Removal of mRNA prior to synthesis of second strand cDNA (1).
- RT-PCR and RT-qPCR: removal of RNA after first strand cDNA synthesis.
- Removal of the poly(A) sequences of mRNA after hybridization with oligo(dT) (2).
- Site-specific cleavage of RNA (3).
- Studies of *in vitro* polyadenylation reaction products (4).

Source

E.coli MRE-600 cells.

Molecular Weight

18.4 kDa monomer.

Definition of Activity Unit

One unit of the enzyme catalyzes the formation of 1 nmol of acid soluble products in 20 min at 37°C.
Enzyme activity is assayed in the following mixture:
20 mM Tris-HCl (pH 7.8), 40 mM KCl, 8 mM $MgCl_2$,
1 mM DTT, 24 µM [^{3}H]-poly(A)·poly(dT), 0.03 mg/ml BSA,
4% (v/v) glycerol.

Storage Buffer

The enzyme is supplied in: 25 mM HEPES-KOH (pH 8.0), 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.1 mg/ml BSA and 50% (v/v) glycerol.

10X Reaction Buffer

200 mM Tris-HCl (pH 7.8), 400 mM KCl, 80 mM $MgCl_2$, 10 mM DTT.

Inhibition and Inactivation

- Inhibitors: metal chelators, SH-blocking reagents.
- Inactivated by heating at 65°C for 10 min.

CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay

No conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 10 units of RNase H with 1 µg of pUC19 DNA for 1 hour at 37°C.

Ribonuclease Assay

No contaminating RNase activity was detected after incubation of 10 units of RNase H with 1 µg of [^{3}H]-RNA for 1 hour at 37°C.

Labeled Oligonucleotide (LO) Assay

No degradation of single-stranded and double-stranded labeled oligonucleotide was observed after incubation with 10 units of RNase H for 1 hour at 37°C.

Quality authorized by:  Jurgita Zilinskiene

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Protocol for Second Strand cDNA Synthesis

1. Perform first strand cDNA synthesis reaction according to recommendations provided for a specific reverse transcriptase.
2. Add the following (on ice) to 20 µl of first strand cDNA synthesis reaction mixture:

10X reaction buffer for DNA Polymerase I*	8 µl
RNase H	0.2 µl (1 u)
DNA Polymerase I (#EP0041)	3 µl (30 u)
Water, nuclease-free (#R0581)	to 100 µl
Total volume	100 µl

* 10X reaction buffer for DNA Polymerase I: 500 mM Tris-HCl (pH 7.5 at 25°C), 100 mM $MgCl_2$, 10 mM DTT.

3. Gently vortex and briefly centrifuge.
4. Incubate at 15°C for 2 hours. Do not let the temperature rise above 15°C.
5. Add 2.5 µl (12.5 u) of T4 DNA Polymerase (#EP0061) and incubate at 15°C for 5 min.
6. Terminate the reaction by adding 5 µl of 0.5 M EDTA, pH 8.0 (#R1021). Phenol/chloroform purified blunt-end cDNA can be used for further cloning related procedures, e.g., adapter ligation, phosphorylation, size fractionation, ligation and transformation.

References

1. Gubler, U., Hoffman, B.J., A simple and very efficient method for generating cDNA libraries, Gene, 25, 263-269, 1983.
2. Davis, R. et al., Tandemly repeated exons encode 81-base repeats in multiple developmentally regulated *Schistosoma mansoni* transcripts, Mol. Cell Biol., 8, 4745-4755, 1988.
3. Donis-Keller, H., Site specific enzymatic cleavage of RNA, Nucleic Acids Res., 7, 179-192, 1979.
4. Goodwin, E.C., Rottman, F.M., The use of RNase H and poly(A) junction oligonucleotides in the analysis of *in vitro* polyadenylation reaction products, Nucleic Acids Res., 20, 916, 1992.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively *for research purposes and in vitro use only.* The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
Please refer to www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.