thermo scientific

PRODUCT INFORMATION

RNase H

Pub. No. MAN0011998
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Lot: _ **Expiry Date: _**

Store at -20 °C

Components	#EN0201	#EN0202
RNase H, 5 U/µL	100 U	500 U
10X Reaction Buffer	1 mL	1 mL

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 detectable degradation was observed after incubation of supercoiled plasmid DNA with RNase H.

Ribonuclease Assay

No detectable degradation was observed after incubation of [^{3}H]-RNA with RNase H.

Labeled Oligonucleotide (LO) Assay

No detectable degradation was observed after incubation of single stranded and doublestranded radiolabeled oligonucleotideswith RNase H.

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.

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