Fermentas
LIFE SCIENCES

**CERTIFICATE OF ANALYSIS**

# RNase H

**#EN0202** 500 u
**Lot:** **Expiry Date:**

Concentration: 5 u/µl

Supplied with: 1 ml of 10X Reaction Buffer

**Store at -20°C**

In total 2 vials. BSA included

ISO 9001 ISO 14001
www.fermentas.com

## 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.

## QUALITY CONTROL ASSAY DATA

### Endodeoxyribonuclease Assay

No detectable 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 in 50 µl of reaction buffer for 1 hour at 37°C.

### Ribonuclease Assay

≥0.5% of the total radioactivity was released into the trichloroacetic acid-soluble fraction after incubation of 10 units of RNase H with 1 µg of [$^3$H]-RNA in 50 µl of reaction buffer for 1 hour at 37°C.

### Labeled Oligonucleotide (LO) Assay

No detectable degradation of a single-stranded or 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

*(continued on back page)*

## 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.