

CERTIFICATE OF ANALYSIS

# Ribonuclease H, *E.coli*

**#EN0201**      100u

**Lot:**                      **Expiry Date:**

Concentration:      5u/μl

Supplied with:      1ml of 10X Reaction Buffer

**Store at -20°C**

In total 2 vials

BSA included: Lot# BSA62-313P

## Description

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

## Source

*E.coli* MRE-600 cells.

## Molecular Weight

18.4kDa monomer.

## Definition of Activity Unit

One unit of the enzyme catalyzes the formation of 1nmol of acid soluble products in 20min at 37°C.

Enzyme activity is assayed in the following mixture:

20mM Tris-HCl (pH 7.8), 40mM KCl, 8mM MgCl<sub>2</sub>,

1mM DTT, 24μM [<sup>3</sup>H]-poly(A)·poly(dT), 0.03mg/ml BSA and 4% (v/v) glycerol.

## Storage Buffer

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

## 10X Reaction Buffer

200mM Tris-HCl (pH 7.8), 400mM KCl, 80mM MgCl<sub>2</sub>, 10mM DTT.

## Applications

- Removal of mRNA prior to the synthesis of the second strand of cDNA (1), see protocol on back page.
- 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).

## Inhibition and Inactivation

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

## 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 Ribonuclease H with 1µg of pBR322 DNA in 50µl of reaction buffer for 1 hour at 37°C.

### Exodeoxyribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 10 units of Ribonuclease H with 1µg of sonicated *E.coli* [<sup>3</sup>H]-DNA in 50µl of reaction buffer for 1 hour at 37°C.

### Ribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 10 units of Ribonuclease H with 1µg of [<sup>3</sup>H]-RNA in 50µl of reaction buffer for 1 hour at 37°C.

Quality authorized by:

 Jurgita Zilinskiene

(continued on back page)

## Protocol for Second Strand cDNA Synthesis

- 1 Synthesize the first strand cDNA using RevertAid™ H Minus First Strand cDNA Synthesis Kit (#K1631), RevertAid™ First Strand cDNA Synthesis Kit (#K1621), or First Strand cDNA Synthesis Kit (#K1611). Stop the reaction by heating at 70°C for 10 minutes.
- 2 Add the following components to 20µl of the first strand cDNA synthesis reaction on ice:

<b>10X reaction buffer for DNA Polymerase I*</b>	8µl
<b>Water, nuclease-free</b> (#R0581)	68.8µl
<b>Ribonuclease H, <i>E.coli</i></b>	0.2µl (1u)
<b>DNA Polymerase I, <i>E.coli</i></b> (#EP0041)	3µl (30u)

- 3 Gently vortex the contents of the tube.
- 4 Incubate at 15°C for 2 hours. **Do not let the temperature rise above 15°C.**
- 5 For blunting of DNA ends, add 2.5µl (12.5u) of T4 DNA Polymerase (#EP0061) and incubate at 15°C for 5 minutes.
- 6 Terminate the reaction by adding 5µl of 0.5M EDTA, pH 8.0, (#R1021).
- 7 Purify blunt-ended dsDNA from step 6 by phenol/chloroform extraction and use for further procedures (e.g., adapter addition, phosphorylation, size fractionation, ligation and transformation).

### Note

- \* 10X reaction buffer for DNA Polymerase I: 500mM Tris-HCl (pH 7.5 at 25°C), 100mM MgCl<sub>2</sub>, 10mM DTT.

## 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.fermentas.com](http://www.fermentas.com) for Material Safety Data Sheet of the product.

## Related Products

- M-MuLV Reverse Transcriptase #EP0351, #EP0352
- RevertAid™ M-MuLV Reverse Transcriptase (*not available in the USA*) #EP0441, #EP0442
- RevertAid™ H Minus M-MuLV Reverse Transcriptase (*not available in the USA*) #EP0451, #EP0452
- DNA Polymerase I, *E. coli* #EP0041, #EP0042
- TrueStart™ Taq DNA Polymerase #EP0611, #EP0612
- Hot Start Taq DNA Polymerase #EP0601, #EP0602
- Taq DNA Polymerase (recombinant) #EP0401, #EP0402  
#EP0403, #EP0404
- Taq DNA Polymerase (native) #EP0281, #EP0282  
#EP0283, #EP0284  
#EP0071, #EP0072
- Pfu DNA Polymerase (native) #EP0571, #EP0572
- Pfu DNA Polymerase (recombinant) #EP0501, #EP0502
- T4 DNA Ligase #EL0011, #EL0012,  
#EL0013, #EL0014,  
#EL0015, #EL0016, #EL0017,  
#EL0331, #EL0334,  
#EL0335, #EL0336, #EL0337
- First Strand cDNA Synthesis Kit #K1611, #K1612
- RevertAid™ First Strand cDNA Synthesis Kit (*not available in the USA*) #K1621, #K1622
- RevertAid™ H Minus First Strand cDNA Synthesis Kit (*not available in the USA*) #K1631, #K1632
- 0.5M EDTA, pH 8.0, molecular biology grade #R1021
- DEPC-treated Water #R0601, #R0603