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ProductInformation

Ribonuclease H
from *Escherichia coli*

Product Number **R 6501**
Storage Temperature -20 °C

Product Description

Enzyme Commission (EC) Number: 3.1.4.34
CAS Number: 9050-76-4
Molecular Weight: 17.6 kDa[1]
Synonym: RNase H

Ribonuclease H from *E. coli* is an endoribonuclease that specifically hydrolyzes the phosphodiester bonds of RNA in RNA:DNA duplexes to generate products with 3'-hydroxyl and 5'-phosphate ends.[1,2,3] RNase H degrades only the RNA component of the DNA-RNA hybrid (RNA that is hydrogen bonded to a complementary DNA strand). Other enzymes in *E. coli* which degrade RNA in the DNA-RNA hybrid are DNA polymerase I and exonuclease III, but these degrade either the RNA or DNA of the hybrids. Ribonuclease H will not cleave single-stranded or double-stranded DNA or RNA.[1,2]

The pH optimum for ribonuclease H is 7.5 to 9.1. The enzyme is activated by Mg^{2+} (2 - 4 mM). RNase H is a sulfhydryl containing enzyme, and is activated by the presence of dithiothreitol and inhibited by N-ethylmalemide.[2]

Ribonuclease H can be used in the following applications:

1. Facilitating the synthesis of double stranded cDNA by removing the mRNA strand of the RNA:DNA duplex produced during the first strand synthesis of cDNA.[4,5]
2. Creating specific cleavages in RNA molecules by using synthetic deoxyoligonucleotides to create local regions of RNA:DNA duplexes.[6]

Precautions and Disclaimer

For Laboratory Use Only. Not for drug, household or other uses.

Procedure

Reaction conditions for a 100 µl reaction:

20 mM HEPES-KOH Buffer, pH 8.0
50 mM KCl
4 mM $MgCl_2$
1 mM DTT
2 µg RNA:DNA Duplex
50 µg/ml BSA
1 Unit Ribonuclease H

Incubate for 20 minutes at 37 °C. Stop the reaction with 1 µl of 0.5 M EDTA.

The volume of the reaction, amount of DNA, units of enzyme, temperature, time, and method of stopping the reaction may be varied .[4,5]

References

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6. Donis-Keller, H., Site specific enzymatic cleavage of RNA. Nucleic Acids Res., **7(1)**, 179-192 (1979).

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