

Thermostable RNase H

REF: EG24201S

Storage Condition

-20°C

Components

Component	Amount
Thermostable RNase H (5 U/μl)	200 U
10× RNase H Reaction Buffer	1 ml

Description

Thermostable RNase H, is an endoribonuclease that remains active at higher temperatures (above 65 °C). Thermostable RNase H can selectively identify and cleave the RNA strand in the RNA:DNA heteroduplex, while the DNA in the heteroduplex remains intact. Thermostable RNase H does not degrade single-stranded or double-stranded RNA or DNA.

Applications

1. Preparation of high-precision RNA structure mapping and site-specific cleavage of RNA.
2. Removal of mRNA in the synthesis of the second strand of cDNA.
3. Removal of Poly(A) tail from mRNA in the presence of Oligo(dT).
4. Component in isothermal amplification experiments.

Definition of Activity Unit

One unit is defined as the amount of enzyme required to produce 1 nmol of ribonucleotides from 40 pmol of a fluorescently labeled 25 base pair RNA:DNA hybrid in a total reaction volume of 50 μl in 20 minutes at 50°C .

Quality Control Assays

Endonuclease Activity

A 20 μl reaction containing 200 ng of supercoiled plasmid and 5 U of Thermostable RNase H incubated for 4 hours at 37 °C results in <10% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

Non-specific Nuclease Activity

A 20 μl reaction containing 15 ng of dsDNA fragments and 5 U of Thermostable RNase H incubated for 16 hours at 37 °C results in no detectable degradation of the dsDNA fragments as determined by agarose gel electrophoresis.

RNase Activity

A 10 μl reaction containing 500 ng of RNA and 5 U of Thermostable RNase H incubated for 1 hour at 37°C results in >90% of the substrate RNA remains intact as determined by agarose.

Residual Host DNA

The product was tested by TaqMan qPCR with primers specific for the *E.coli* 16S rDNA , and the results show that the *E.coli* genome residues less than 10 copies/5 U.

Notice

1. The reaction buffer of Thermostable RNase H contains MgCl₂. When using Thermostable RNase H at high temperatures for the target RNA:DNA heteroduplex and other single-stranded RNA (total RNA), it is recommended to control the reaction time and temperature to reduce the degradation of single-stranded RNA by metal ions.
2. Thermostable RNase H can be inactivated by adding protease K or excessive EDTA.
3. The optimal reaction temperature is >65°C , and the maximum is 95°C . The activity of Thermostable RNase H at 65°C is 3 to 4 times that at 37°C .
4. For your safety and health, please wear lab coats and disposable gloves for operation.