Thermostable RNase H

(Thermus thermophilus)

Cat. No.	size
E1325-01	500 units
E1325-02	2500 units

Source:

E.coli strain carrying the RNase H gene from *Thermus thermophilus*.

Unit Definition:

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

Optimal reaction temperature: 65°C

Inactivation temperature: no

Package contents:

Thermostable RNase H (5u/µl)

10x RNase H reaction buffer

Storage Conditions:

Store at -20°C.

Description:

Thermostable RNaseH is an enzyme that specifically degrades only the RNA strand of an RNA:DNA hybrid, leaving the DNA strand and any unhybridized RNA intact. Unlike E. coli RNase H, Tth RNase H is highly active and stable at high temperatures.

Applications:

- RNA structure mapping and site-specific RNA cleavage
- Removal of poly (A) tails from mRNA hybridized with oligo (dT)
- Removal of mRNA during second-strand cDNA synthesis
- Component of isothermal amplification methods

Storage Buffer:

20 mM Tris-HCl (pH 8.0 at 22°C), 100 mM KCl, 1 mM dithiothreitol, 0.1 mM EDTA, 0.5% Igepal, 0.5% Tween 20 and 50% (v/v) glycerol.

10x reaction buffer:

500 mM Tris-HCl (pH 8.3 @ 25°C), 750 mM KCl, 30 mM $MgCl_2$, 100 mM DTT

Standard Reacton Protocol (for 50 µl volume):

1. Mix the following reaction components:

Reaction components:	Final volume: 50 µl
RNA:DNA duplex	1 µg
10x RNase H Reaction Buffer	5 µl
Thermostable RNase H (5u/µl)	1 µl
Nuclease-free H_2O	up to 50 µl

2. Incubate at 50°C for 20 minutes (A higher temperature can be set. Use a temperature below the melting point of the RNA:DNA duplex).
3. The reaction can be stopped with addition of 0,5 µl of 0.5 M EDTA.

Quality Control:

All preparations are assayed for contaminating single- and double-stranded DNase, endonuclease, RNase III and nonspecific RNase activities. Typical preparations are greater than 90% pure, as judged by SDS polyacrylamide gel electrophoresis.

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