RNase H

FB02517010 100 Units
FB02517050 500 Units
Store at -25°C to -15°C

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.
- 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).
- Site-specific cleavage of RNA.
- Studies of *in vitro* polyadenylation reaction products.

Kit Contents

Reagents	100 Units	500 Units
RNase H, 5 U/µL	20 µL	100 µL
10X Reaction Buffer	1 mL	1 mL

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.

Protocol for Second Strand cDNA Synthesis

1. Perform first strand cDNA synthesis reaction according to recommendations provided for a specific reverse transcriptase.
2. Prepare the following reaction mixture on ice:

Component	100 µL Reaction
First strand cDNA synthesis reaction mixture	20 µL
RNase H	0.2 µL (1U)
10X reaction buffer for DNA Polymerase I	8 µL
DNA Polymerase I	3 µL (30 U)
Water, nuclease-free	to 100 µL

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 and incubate at 15°C for 5 min.
6. Terminate the reaction by adding 5 µL of 0.5 M EDTA, pH 8.0. Phenol/chloroform purified blunt-end cDNA can be used for further cloning related procedures, e.g., adapter ligation, phosphorylation, size fractionation, ligation and transformation.