RNase H

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RNase H

RNase H is a 18.9 kDa recombinant endoribonuclease purified from an *Escherichia coli* strain, which over-expresses cloned RNase H gene (*rnh*). The enzyme hydrolyses specifically the phosphodiester bonds of RNA hybridized to DNA and produces 5´ phosphate-terminated oligoribonucleotides and single-stranded DNA. RNase H does not degrade single and double-stranded DNA or unhybridized RNA. It is a key enzyme in the removal of mRNA after first-strand cDNA synthesis. Treating cDNA with RNase H prior to PCR can improve sensitivity as RNA bonded to the cDNA template may prevent binding of the amplification primers in a PCR reaction. RNase H treatment is often necessary when amplifying longer, full-length cDNA targets. In addition, RNase H is useful for the removal of poly(A) tails on mRNAs after hybridization with oligo(dT) and also for the site-specific enzymatic cleavage of RNA.

Applications

- → Removal of RNA after first strand cDNA synthesis (RT-PCR and qRT-PCR)
- → Removal of mRNA prior to synthesis of second strand cDNA
- → 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

10x RNase H Reaction Buffer

200 mM Tris-HCl (pH 8.4), 500 mM KCl, 50 mM $MgCl_2$, 200 mM DTT

Usage

- → Use 5 U of enzyme to remove RNA from a RNA:DNA duplex after reverse transcription in a 20 µl reaction. If 50 µl reaction is desired, the use of 12.5 U of enzyme is recommended.
- → The reaction mixture should be incubated at 37°C for 20 minutes.

Additional information

- → The activity of RNase H is inhibited by metal chelators (e.g. EDTA) and sulfhydryl SH-blocking reagents.
- → Inactivate enzyme by heating at 65°C for 10 min.

Quality control

RNase H is >90% pure as judged by SDS polyacrylamide gel. The absence of DNase, RNase and protease activity has been confirmed using the relevant procedures.

Unit definition

One unit catalyses the hydrolysis of 1 nmol of RNA in [^{3}H]-labeled poly(A)×poly(dT) to acid-soluble ribonucleotides in a total reaction volume of 50 µl in 20 min at 37°C in 1x RNase H Reaction Buffer.

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Components	RT34-025 250 U	RT34-125 1250 U	RT34-S 40 U
RNase H 5 U/µl	50 µl	250 µl	8 µl
10x RNase H Reaction Buffer	150 µl	750 µl	24 µl

Storage & shipping

Storage conditions
Store at -20°C.

Shipping conditions
Shipping on dry or blue ice.

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