August 2023

Quick-Start Protocol

RNase H MBG

RNase H MBG (cat. nos. RT34-025 and RT34-125) 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 MBG 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 MBG 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 MBG treatment is often necessary when amplifying longer, full-length cDNA targets. In addition, RNase H MBG 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. RNase H MBG must be shipped on dry ice and stored at –20°C.

Further information

- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

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

- RNase H MBG is >90% pure as judged by SDS polyacrylamide gel. The absence of DNase, RNase, and protease activity has been confirmed using the relevant procedures.
- 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 minutes at 37°C in 1x RNase H Buffer.

Procedure

1. 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.
2. The reaction mixture should be incubated at 37°C for 20 min.

Document Revision History

Date	Changes
08/2023	Initial release

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