MB085_IFU_EN_V2401

NZY RNase H (*E. coli*)

Catalogue number	Presentation
MB08501	250 U
MB08502	1250 U

Description

NZY RNase H is a recombinant endoribonuclease purified from an *Escherichia coli* strain that over-expresses the cloned RNase H gene (rnh). The enzyme specifically hydrolyses the phosphodiester bonds of RNA which is hybridized to DNA to produce 5´ phosphate-terminated oligoribonucleotides and single-stranded DNA. RNase H (*E. coli*) 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 cDNA-RNA hybrids in the amplification reaction may prevent binding of the amplification primers. RNase H treatment is often necessary when amplifying longer, full-length cDNA targets. Additionally, RNase H is useful for removing poly(A) tails from mRNAs after hybridization with oligo(dT) and for site-specific enzymatic cleavage of RNA.

Shipping & Storage Conditions

This product is shipped in dry ice. Upon receipt, store all components at -85 to -15 °C in a constant temperature freezer. Stored as specified, the product will remain stable until the expiry date.

Components

	MB08501 (250 U)		MB08502 (1250 U)	
COMPONENT	**TUBES**	**VOLUME**	**TUBES**	**VOLUME**
NZY RNase H (*E. coli*)	1	50 µL	5	50 µL
10x Reaction buffer for RNase H	1	750 µL	2	750 µL

Specifications

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 1× Reaction Buffer.

Enzyme concentration: 5 U/µL.

Inhibition & Inactivation: NZY RNase H (*E. coli*) is inhibited in the presence of metal chelators (e.g. EDTA) and sulfhydryl SH-blocking reagents. The enzyme is inactivated at 65 °C for 10 min.

Standard Protocol

Recommendations before starting

- Nucleic acid manipulation: To ensure the integrity and purity of RNA, follow these precautions:
  - Maintain aseptic conditions: Always wear gloves, change them if suspected of contamination. We recommend using RNase-free plasticware/reagents, filtered tips and work in an RNase-free area. The RNase & DNase Cleaner (NZYtech, Cat. No. MB463) can help removing RNases from surfaces and materials. Designate a dedicated area and equipment solely for RNA work.
  - Store RNA at -85 °C to -65 °C and avoid subjecting RNA to multiple freeze/thaw cycles. Perform all reaction steps on ice.
  - Assess the RNA purity concerning contaminants by examining the ratio of absorbance at 260 nm and 280 nm (A260/A280). Ideally, pure RNA should exhibit an A260/A280 ratio within the range of 1.9-2.1 in a 10 mM Tris-HCl buffer at pH 7.5.
- Reagent usage:
  - The reaction buffer provided already contains Mg^{2+} at the optimal concentration for NZY RNase H (*E. coli*) activity. In extremely rare cases, the addition of extra Mg^{2+} for concentration adjustments may be considered.
- Handling instructions: Keep all reagents on ice while setting up the reactions. Minimize the duration of RNA exposure to ice

Procedure for cleaving RNA strand within an RNA:DNA hybrid

1. On ice, in a sterile, nuclease-free microcentrifuge tube, prepare a reaction mixture, combining the following components:
   Note: *If setting up more than one reaction, prepare a reaction mixture volume 10% greater than the total required for the number of reactions to be performed.*

COMPONENT	1 REACTION VOLUME
RNA:DNA hybrid	2 µg
10x Reaction buffer for RNase H	10 µL
NZY RNase H (*E. coli*)	1 µL (5 units)
FINAL VOLUME =	100 µL

2. Mix gently and centrifuge briefly.
3. Incubate at 37 °C for 20 min.
4. Inactivate the reaction by heating at 65 °C for 10 min, or by adding 1 µL of EDTA at 0.5 M.
5. Store the reaction product at - 85 °C to -65 °C or proceed to downstream applications.

Quality control

Purity

NZY RNase H (*E. coli*) is >90% pure as judged by SDS polyacrylamide gel electrophoresis followed by Coomassie Blue staining.

Genomic DNA contamination

The product must comply with internal standards of DNA contamination as evaluated through real-time qPCR.

Nucleases assay

To test for DNase contamination, 0.2-0.3 µg of pNZY28 DNA are incubated with 5 U of NZY RNase H for 14-16 h at 37 °C. To test for non-specific RNase activity, 1 µg of RNA is incubated with 5 U of the enzyme for 1 h at 37°C. Following incubation, the nucleic acids are visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the nucleic acids. Similar tests are performed with reaction buffer.

Functional assay

NZY RNase H (*E. coli*) and respective buffer are tested for performance in a RT-qPCR experiment by adding 1 µL of NZY RNase H (*E. coli*) to 20 µL of the RT product reaction.