

## 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

The product can be shipped from dry ice to blue 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

COMPONENT	MB08501 (250 U)		MB08502 (1250 U)	
	TUBES	VOLUME	TUBES	VOLUME
NZY RNase H ( <i>E. coli</i> )	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 [<sup>3</sup>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<sup>2+</sup> at the optimal concentration for NZY RNase H (*E. coli*) activity. In extremely rare cases, the addition of extra Mg<sup>2+</sup> 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 ( <i>E. coli</i> )	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.

For life science research only. Not for use in diagnostic procedures.