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RNase H

1 Components

Component	HY-KE7058-100 U
RNase H	100 U
10× Reaction Buffer	100 μL

2 Introduction

RNase H is an endoribonuclease expressed and purified from *Escherichia coli* and can specifically hydrolyze RNA in DNA-RNA hybrid strands.

3 Properties

Source	*E.coli* MRE-600 cells.
Molecular weight	18.4 kDa monomer
Storage buffer	10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 200 μg/mL BSA, 50% (v/v) glycerol, (pH 7.4, 25°C).
10× Reaction Buffer	500 mM Tris-HCl, 750 mM KCl, 30 mM $MgCl_2$, 100 mM DTT, (pH8.3, 25°C).
Enzyme activity	5 U/μL
Unit Definition	One unit is defined as the amount of enzyme required to produce 1nmol of ribonucleotides from 20 picomoles of afluorescently labelled 50 base pair RNA-DNA hybrid in a total reaction volume of 50 μL in 20 minutes at 37°C.

4 General Protocol

1. Protocol for removal RNA from DNA-RNA hybrid strands

1) Add the following (on ice) to 20 μL of first strand cDNA synthesis reaction mixture:

Components	Adding amount
Deionized water (Nuclease-free)	17.8 μL
10× Reaction Buffer	2 μL
RNase H	0.2 μL

2) Gently vortex and briefly centrifuge.

3) Incubate at 37°C for 1 h.

4) Terminate the reaction by adding 2.5 μL of 0.5 M EDTA (pH 8.0).

Note: The conditions for removing RNA from the DNA-RNA hybrid strand can be carried out by referring to the above conditions. The pH range of the RNase H reaction is approximately 7.5-8.3.

2. Protocol for removal of RNA in the hybrid strand and synthesis of the second strand of cDNA

Need to prepare your own reagent DNA Polymerase I (HY-P2937).

1) Add the following (on ice) to 20 μL of first strand cDNA synthesis reaction mixture:

Components	Adding amount
Deionized water (Nuclease-free)	17.8 μL
10× Reaction Buffer	2 μL
RNase H	0.2 μL
DNA Polymerase I	30 U

2) Gently vortex and briefly centrifuge.

3) Incubate at 15°C for 2 h.

Note: Do not let the temperature rise above 15°C.

4) Terminate the reaction by adding 5 μL of 0.5 M EDTA (pH 8.0).

5 Storage

-20°C, 2 years

6 Precautions

1. The enzyme should be stored in an ice box or on an ice bath when in use. After use, it should be stored at -20°C immediately.
2. It is normal for white precipitate to appear when thawing 10× Reaction Buffer. The white precipitate is DTT in the Buffer and can be mixed before use.
3. This product is for R&D use only, not for drug, household, or other uses.
4. For your safety and health, please wear a lab coat and disposable gloves to operate.