

Thermostable RNase HIII

Please read the manual carefully before use

Cat. No. LH201

Version No. Version 1.0

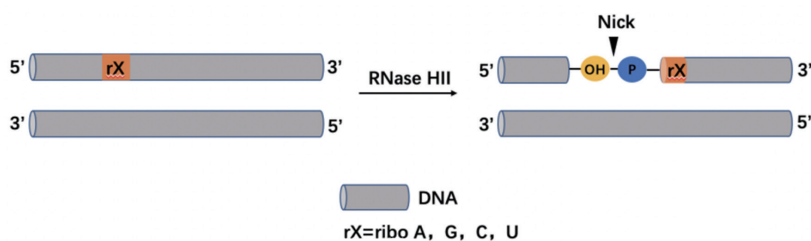
Storage: at -18°C or below for two years

Concentration: 2 U/μl

Description

Thermostable RNase HIII is an endoribonuclease derived from *Pyrococcus abyssi* (*P.a.*), genetically engineered and expressed in *Escherichia coli* (*E. coli*). This enzyme cleaves the 5' phosphodiester of ribonucleotides within the context of dsDNA, generating 5' phosphate and 3' hydroxyl ends. It exhibits very low activity against single-stranded RNA (ssRNA) and no cleavage activity against double-stranded DNA (dsDNA) or single-stranded DNA (ssDNA). The enzyme is highly thermostable, retaining its activity even after heating at 95°C for 45 minutes. It is widely used in applications such as RNase HIII-dependent PCR (rhPCR), loop-mediated isothermal amplification (LAMP), and degradation of the RNA portion of Okazaki fragments.

Schematic Diagram of the RNase HIII Reaction Principle



Thermostable RNase HIII cleaves the 5' phosphodiester of ribonucleotides within the context of dsDNA, generating 5' phosphate and 3' hydroxyl ends.

Kit Contents

Component	LH201-01	LH201-02
Thermostable RNase HIII	50 units	250 units
2×RNase HIII Reaction Buffer	0.5 ml	1.5 ml

Unit Definition

One unit is defined as the amount required to generate a fluorescence signal equivalent to nicking 100 pmol of a synthetic double-stranded DNA substrate containing a single ribonucleotide within 30 minutes at 60 °C in a reaction buffer consisting of 20 mM Tris-HCl, 10 mM (NH₄)₂SO₄, 10 mM KCl, 2 mM MgSO₄, 0.1% Triton X-100, pH 8.8 (at 25 °C).

Enzyme Storage Buffer

20 mM Tris-HCl, 100 mM NaCl, 1 mM DTT, 1 mM EDTA, 50% glycerol, pH8.0@25°C

Notes

- Thermostable RNase HIII is extremely heat-resistant. Its activity remains unchanged after heating at 95 °C for 30 minutes, and therefore cannot be inactivated by heat treatment. To fully inactivate the enzyme after the reaction, add SDS to a final concentration of 0.1% to the reaction mixture.
- When processing Okazaki fragments, thermostable RNase HIII preferentially generates a nick at the 5' side of the ribonucleotide at the RNA/DNA junction.



Applications

1. High-sensitivity probe-based LAMP detection.
2. RNase HII-dependent PCR (rhPCR).
3. Removal of ribonucleotides misincorporated during polymerase chain elongation.
4. Degradation of the RNA portion of Okazaki fragments.

FAQs & Solutions

1. Heating does not inactivate thermostable RNase HII. How can the enzyme be inactivated?
Add SDS to the reaction mixture to a final concentration of 0.1% to fully inactivate thermostable RNase HII.
2. Which end of the ribonucleotide does thermostable RNase HII cleave?
Thermostable RNase HII nicks the 5' phosphodiester of ribonucleotides within the context of dsDNA, generating 5' phosphate and 3' hydroxyl ends.
3. How should the 2× RNase HII Reaction Buffer be used?
Thermostable RNase HII can be used in various reaction systems for the applications mentioned above. The 2× RNase HII Reaction Buffer is the standard reaction buffer for testing its activity and can be used as a control reaction buffer.

Quality Control

Item	Specification
Appearance	Colorless and transparent
Molecular Weight	25.4 kDa
Isoelectric Point	9.0
Purity	≥95% (SDS-PAGE)
Enzyme Activity	2 U/μl
Working Temperature	Optimal 80°C (effective range 50-90°C)
RNase Activity	Not detected
DNase Activity	Not detected
Nickase Activity	Not detected

For research use only, not for clinical diagnosis.

Version number: V1.0-202512

Service telephone +86-10-57815020

Service email custserv@transgenbiotech.com

