Thermostable RNase HII

REF: EG24212S

Storage Condition

-20℃

Components

Component	Amount
Thermostable RNase HII (2 U/μl)	100 μl
HII Dilution Buffer	2×1 ml
10× RHII Buffer	1 ml

Description

This product is derived from the extremely thermophilic bacterium *Pyrococcus abyssi* (*P.abyssi*) and obtained through recombinant expression and purification. Thermostable RNase H is an endonuclease that specifically recognizes and cleaves the RNA strand in RNA/DNA heteroduplexes, without cleaving sSDNA, dsDNA, and exhibits minimal cleavage activity towards single-stranded RNA. Thermostable RNase HII cleaves at the 5' end of ribonucleotide residues, generating a 5'-phosphate group and a 3'-hydroxyl group after cleavage. This Thermostable RNase HII exhibits optimal activity within the temperature ranging from 60 to 80℃ , remains active between 37℃ and 95℃ , and exhibits low activity at room temperature. The product is extremely thermostable, with almost no activity loss following incubation at 95 ℃ for 15 minutes, making it compatible with various PCR reaction systems.

Definition of Activity Unit

One unit is defined as the amount of enzyme required to cleave 100 pmol of a chimeric DNA/RNA hybrid substrate containing a single ribonucleotide at 60℃ for 30 minutes.

Recommended reaction conditions

1× RHII Buffer;
Incubation at 60℃ .

Heat Inactivation

Final Concentration: 1%SDS，Incubation at 95℃ for 15 minutes.

Applications

1. RNase HII dependent PCR (rhPCR)。
2. Reduce or eliminate primer dimers in PCR reactions.
3. Improve the accuracy of multiplex PCR products.
4. Differentiating paralogous genes.
5. SNP and rare allele detection.
6. Degradation of the RNA portion of Okazaki fragments.
7. LAMP high sensitivity probe detection.
8. Generation of a double-stranded break at the site of an incorporated ribonucleotide when used with T7 Endo I.

Quality Control Assays

Protein Purity

The enzyme is ≥95% pure as determined by SDS-PAGE analysis using Coomassie Blue staining.

Endonuclease Activity

A 20 μl reaction containing 200 ng of supercoiled plasmid and 2 U of Thermostable RNase HII incubated for 4 hours at 37℃ results in <20% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

Non-specific Nuclease Activity

A 20 μl reaction containing 15 ng of dsDNA fragments and 2 U of Thermostable RNase HII incubated for 16 hours at 37 ℃ results in no detectable degradation of the dsDNA fragments as determined by agarose gel electrophoresis.

RNase Activity

A 10 μl reaction containing 500 ng of RNA and 2 U of Thermostable RNase HII incubated for 1 hour at 37℃ results in >90% of the substrate RNA remains intact as determined by agarose.

Residual Host DNA

Using the third method of the determination of exogenous DNA residue in General Chapter 3407 of ChP(2020) Volume IV, the residual amount of Escherichia coli host cell DNA in this product is less than 1 copy per 2 units.

Protocol

1. Take the rhPCR workflow as an example:

(1) As an example of use in conjunction with our Taq-Plus PCR Master Mix (2×) (REF: EG15117M):

Reagent	Amount
upstream primer (10 μM)	1 μl
downstream primer (10 μM)	1 μl
Template DNA	x μl
Taq-Plus PCR Master Mix (2×)	25 μl
Thermostable RNase HII	20~200 mU
ddH_2O	Up to 50 μl

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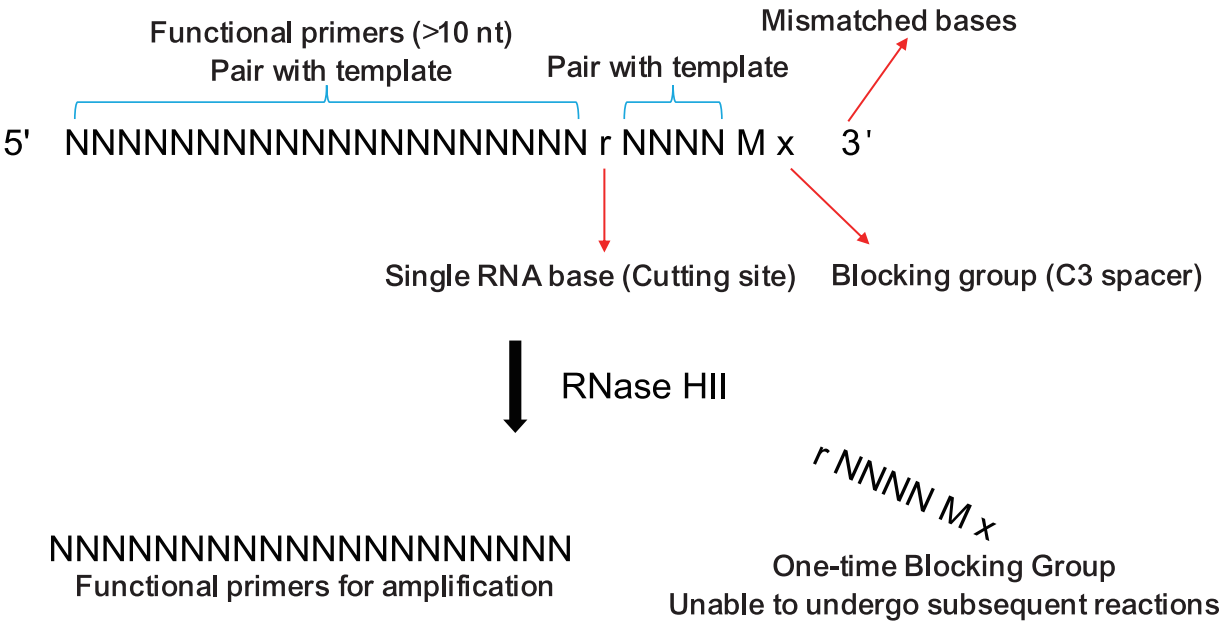
(2) Mix gently and spin down.
(3) PCR Reaction Setup

Step	Temperature	Time
Initial Denaturation	95℃	3~5 min
Denaturation	95℃	30 s
Annealing	55~65℃	30 s
Extension	72℃	30~60 s/kb
Final Extension	72℃	5 min

30~35 Cycles

2. rhPCR primer design



Notice

1. When used in RNase HII-dependent PCR (rhPCR), DNA primers with blocked 3′ ends are required.
2. RNase HII remains active across 37~80℃ , with the amount adjustable depending on the application.
3. RNase HII is compatible with most PCR systems. RNase HII maintains activity in buffers containing 1~6 mM Mg^{2+}.

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