Product Description

AcuGenix™ RNase H II is a thermostable riboendonuclease that specifically cleaves the phosphodiester bond at the 5' side of a ribonucleotide within a DNA/RNA heteroduplex, generating a 3'-hydroxyl terminus and a 5'-phosphate group. Unlike RNase H1, this enzyme requires a minimum of one ribonucleotide embedded within a DNA strand for activity, and does not cleave single-stranded or double-stranded RNA or DNA. AcuGenix™ RNase H II is suitable for use in RNase H-dependent amplification systems, including LAMP-based assay formats.

Size

200 mU/μL

Components

1. RNase H II (200 mU/μL)
2. Bst2.0 HS (8 U/μL) (Sold separately)
3. LAMP Master Mix Buffer II (Sold separately)

Unit Definition

One unit is defined as the amount of enzyme required to cleave 1 nmol of a synthetic duplex substrate containing a single ribocytidine (rC) residue per minute at 70°C, in a buffer containing 10 mM NaCl, 0.01% Triton X-100, 10 μg/mL BSA, and 4 mM $MgCl_2$.

Storage

Store at -20±5°C. Thaw on ice, mix gently, and briefly spin down before use. Avoid repeated freeze-thaw cycles. *If precipitation occurs during frozen storage or after thawing, this is a normal phenomenon; allow the product to equilibrate to room temperature and mix gently before use.

Quality Control

1. Activity, stability, and performance consistency in RNase H II-assisted LAMP assays.
2. Free of exogenous ribonuclease and endonuclease activity; no exogenous deoxyribonuclease contamination.

Prepare Reaction Mix

Components	25 μL System	50 μL System	Final Concentration
Bst2.0 HS (8 U/μL)	1 μL	2 μL	0.32 U/μL
LAMP Master Mix Buffer II	12.5 μL	25 μL	1×
RNase H II (200 mU/μL)[1]	0.125 μL	0.25 μL	0.5 mU/μL
10×Primer Mix[2]	2.5 μL	5 μL	1×
10×Probe Mix[3]	2.5 μL	5 μL	1×
Template[4]	—	—	—
ddH_2O	To 25 μL	To 50 μL	—

1. The amount of RNase H II required may vary by assay. The recommended adjustment range for a 50 μL reaction is 4-400 mU.
2. 10×Primer Mix: 16 μM FIP, 16 μM BIP, 2 μM F3, 2 μM B3, 4 μM LoopF, and 4 μM LoopB in TE buffer.
3. 10×Probe Mix: 1 μM probe primer in TE buffer.
4. For optimal amplification, add an appropriate amount of template: plasmid DNA 10 fg-1 ng, genomic DNA 1 ng-100 ng, or appropriately prepared DNA extract.

Reaction Conditions

Incubate at 60-65°C for 30-60 min, with fluorescence acquisition every 1 min.

LAMP Primer-Probe Design Example

Design probe primers based on the standard 4-6 LAMP primer set. The probe is positioned between the F1 and B1 sequences.

Duplex conformation prior to cleavage:

5' CTGGGAACTCTCTTCCT/rC/TGGCGT 3'

3' GACCCTTGAGAGAAGGAGACCGCA 5'

Cleavage products:
5′ CTGGGAACTCTCTTCCT-OH 3′
5′ Phos/rC/TGGCGT 3′
3′ GACCCTTGAGAGAAGGAGACCGCA 5′