Cat.:FE201

AcuGenix™ RNase H II (Glycerol-Free)

V01

Product Description

AcuGenix™ RNase H II (Glycerol-Free) is a ribonuclease that specifically hydrolyzes the phosphodiester bonds of RNA in DNA-RNA hybrid strands, but does not hydrolyze phosphodiester bonds in single-stranded or double-stranded DNA or RNA. The mechanism of action of this enzyme involves cleaving the phosphodiester bond linking the RNA base to the DNA base in the 5′ direction, resulting in the formation of a 3′ hydroxyl group and a phosphate group at the 5′ end of the RNA. AcuGenix™ RNase H II (Glycerol-Free) is a specialized enzyme formulated to meet the specific requirements of lyophilized reagents; it can be used in PCR amplification systems dependent on ribonuclease H, as well as in reaction systems such as qPCR and LAMP.

Size

200 mU/μL

Unit Definition

One activity unit is defined as the amount of enzyme required to cleave 1 nmol of a synthetic double-stranded substrate containing a single rC (ribonucleic acid) molecule 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. Vortex before use. Avoid freeze-thaw cycles.

Quality control

1. Functional testing: sensitivity, specificity, reproducibility of LAMP.
2. No exogenous nuclease activity, no exogenous endo/exonuclease contamination.
3. DNA sequencing of GC-rich templates and nanogram-level templates.

Protocol

Using LAMP isothermal amplification as an example, please prepare the solution on ice to maintain a low temperature:

Component	Volume per Reaction	Concentration in Master Mix
2×LAMP Premix Buffer	25 μL	1×
8 U/μL Bst 2.0 HS (DG)	2 μL	16 U
10×Primer Mix [1]	5 μL	1×
200 mU/μL RNase H Ⅱ (DG) [2]	0.125 μL	25 mU
Template	——	——
ddH_2O	To 50 μL	——

1.10× Primer Mix: 16 μM FIP, 16 μM BIP, 2 μM F3, 2 μM B3, 4 μM LoopF, 4 μM LoopB, and 1 μM RNase H II Probe Primer in TE Buffer.

2.The amount of RNase H II required may vary depending on the reaction system and should be adjusted according to the specific experiment. For a 50 μL reaction volume, the recommended dosage range is 4–400 mU.

Reaction Programe

65℃,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′ GACCCTTGAG AGAAGGAGACCGCA 5′

Cleavage products:

5′ CTGGGAACTCTCTTCCT-OH 3′

5′ Phos/rC/TGGCGT 3′

3′ GACCCTTGAGAGAAGGAGACCGCA 5′

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