AcuGenix™ Hot Start RT-LAMP Probe Master Mix-V2 V01

Product Description

AcuGenix™ Hot Start RT-LAMP Probe Master Mix-V2 adopts the probe-based isothermal amplification method and can be used for qualitative detection of RNA in samples (e.g., viruses). AcuGenix™ Bst Hot Start DNA Polymerase-V2 is derived from the DNA polymerase of *Bacillus stearothermophilus*. It exhibits 5'→3' DNA polymerase activity and strong strand displacement capability, while lacking 3'→5' exonuclease activity. Compared with the large fragments of wild-type Bst DNA polymerase, this enzyme significantly enhances the amplification speed and yield. It is obtained through reversible modification technology based on genetically engineered Bst 2.0 DNA polymerase. The activity of this enzyme can be completely inactivated at room temperature, allowing reactions to be initiated under ambient conditions. This suppresses non-specific amplification and improves reaction efficiency. Additionally, AcuGenix™ Bst Hot Start DNA Polymerase-V2 does not require separate activation steps.

Components

1. AcuGenix™ Bst Hot Start DNA Polymerase-V2 (8 U/μL)
2. 2×RT-LAMP Premix Buffer Ⅱ
3. Neoscript RTase (200 U/μL)
4. RNase HⅡ (50 mU/μL)

Storage

Store at -20±5℃. Vortex before use. Avoid freeze-thaw cycles.

Prepare Reaction Mix

Components	25 μL System	50 μL System	Final Concentration
AcuGenix™ Bst Hot Start DNA Polymerase-V2 (8 U/μL)	1 μL	2 μL	0.32 U/μL
2×RT-LAMP Premix Buffer Ⅱ	12.5 μL	25 μL	1×
Neoscript RTase (200 U/μL)	0.375 μL	0.75 μL	3 U/μL
RNase HⅡ (50 mU/μL)	0.25 μL	0.5 μL	0.5 mU/μL
10×Primer Mix [1]	2.5 μL	5 μL	1×
10×Probe Mix [2]	2.5 μL	5 μL	1×
Template RNA [3]	——	——	——
ddH2O	To 25 μL	To 50 μL	——

1. Recommended primer concentration: The 10×Primer Mix comprises 16 μM FIP, 16 μM BIP, 2 μM F3, 2 μM B3, 4 μM LoopF, and 4 μM LoopB. Concentration gradient testing can be carried out to determine the optimal primer dosage. It is recommended that primers be prepared with TE Buffer.
2. 10×Probe Mix : 1 μM probe primer in TE Buffer.
3. Different types of RNA templates have different copy numbers of target genes. When necessary, gradient dilution can be performed to determine the optimal quantity of RNA template to be added.

Reaction Conditions

60-65℃, 30-60 minutes (Collect fluorescence per minute).

Quality control

1. Functional testing: sensitivity, specificity, reproducibility of RT-LAMP.
2. No exogenous nuclease activity, no exogenous endo/exonuclease contamination.

Q & A

RT-LAMP amplification products are scarce or non-existent:

1. Elevate the quantity of the template and the concentration of the enzyme.
2. Substitute the primers. It is advisable to design multiple primer sets and conduct screening for the optimal primers via preliminary experiments. For primer design, refer to http://primerexplorer.jp/e/.
3. The use of Loop Primers is recommended to enhance amplification efficiency and reduce amplification time.

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