

Product Description

AcuGenix™ Hot Start Colorimetric RT-LAMP Master Mix (Red-to-Yellow)-V2 employs a visual method for qualitative detection of DNA in samples (such as viruses). AcuGenix™ Bst Hot Start DNA Polymerase-V2 is derived from *Bacillus stearothermophilus* DNA Polymerase, featuring 5'→3' DNA polymerase activity and strong strand displacement capability, without 3'→5' exonuclease activity. Compared to the large fragment of wild-type Bst DNA polymerase, this enzyme can effectively enhance amplification speed and yield. Obtained through reversible modification technology based on Bst 2.0, AcuGenix™ Bst Hot Start DNA Polymerase-V2 can completely inhibit enzyme activity at room temperature, allowing reactions to be established at room temperature, preventing nonspecific amplification, and improving reaction efficiency. This reagent utilizes an optimized LAMP visual method-specific Buffer. Based on the principle of pH value reduction during the amplification reaction process, the reagent's color change is used to interpret the results. The reagent is purple-red before the reaction, and the positive well turns yellow after the reaction, while the negative well remains purple-red. Additionally, AcuGenix™ Bst Hot Start DNA Polymerase-V2 does not require a separate activation step.

Components

1. AcuGenix™ Bst Hot Start DNA Polymerase-V2 (8 U/μL)
2. 2×RT-LAMP Premix Buffer (RY)
3. Neoscript RTase (200 U/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 (RY)	12.5 μL	25 μL	1×
Neoscript RTase (200 U/μL)	0.375 μL	0.75 μL	3 U/μL
10×Primer Mix ¹	2.5 μL	5 μL	1×
Template ²	--	--	--
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. In order to achieve better amplification results, appropriate templates are added.

Reaction Conditions

Incubate at a temperature range of 60-65℃ for a duration of 30-60 minutes, and acquire fluorescence data at one-minute intervals. After the reaction is completed, allow the sample to cool to ambient temperature before analyzing the results.

Quality control

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

Q & A

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.