Cat.:HW206-R02

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

Product Description

Loop-mediated isothermal amplification (LAMP) is a nucleic acid amplification technology. This kit adopts the dye-based isothermal amplification method and can be used for the qualitative detection of DNA in samples (e.g., viruses). AcuGenix™ Bst Hot Start DNA Polymerase-V2 is derived from Bacillus stearothermophilus DNA Polymerase I. Through genetic engineering techniques, it retains the 5′→3′ DNA polymerase activity while eliminating the 5′→3′ exonuclease activity and exhibits strand displacement capability. It is suitable for DNA strand displacement reactions and LAMP (Loop-mediated isothermal amplification). AcuGenix™ Bst Hot Start DNA Polymerase-V2 has been genetically modified to improve its sensitivity, and compared to the wild-type Bst DNA polymerase and long fragments, this enzfiyme can effectively increase the amplication speed and yield. AcuGenix™ Bst Hot Start DNA Polymerase-V2 is a thermosensitive isothermal amplification enzyme obtained via reversible modification technology based on genetically engineered Bst 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.

Components

1. 8 U/μL AcuGenix™ Bst Hot Start DNA Polymerase-V2
2. 200 U/μL Neoscript Reverse Transcriptase
3. 2×RT-LAMP Premix Buffer Ⅱ
4. 50×RT-LAMP Fluorescent Dye Ⅱ

Storage

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

Prepare Reaction Mix

Components	25 μL System	50 μL System	Final Concentration
8 U/μL AcuGenix™ Bst Hot Start DNA Polymerase-V2	1 μL	2 μL	0.32 U/μL
200 U/μL Neoscript Reverse Transcriptase	0.375 μL	0.75 μL	3 U/μL
2×RT-LAMP Premix Buffer Ⅱ	12.5 μL	25 μL	1×
50×RT-LAMP Fluorescent Dye Ⅱ	0.5 μL	1 μL	1×
10×Primer Mix [1]	2.5 μL	5 μL	1×
Template RNA [2]	——	——	——
ddH_2O	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. 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. This product functions as a pH-sensitive indicator reagent. High - concentration Tris-HCl buffer systems should be avoided in sample elution solutions. The use of strong acid or strong alkali nucleic acid release agents for sample processing should be avoided. If such reagents are unavoidably used, it is advisable to adjust the pH to neutral.

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.

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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.