Cat.:HW205-P01

AcuGenix™ Hot Start LAMP Probe Master Mix-V2

V01

Product Description

Loop mediated isothermal amplification (LAMP) is a nucleic acid amplification technique. This kit uses dye based isothermal amplification and can be used for qualitative detection of DNA in samples such as viruses. AcuGenix™ Bst Hot Start DNA Polymerase-V2 is derived from Bacillus stearothermophilus DNA polymerase. Compared with the wild-type Bst DNA polymerase large fragment, this enzyme can effectively improve amplification speed, yield, etc. AcuGenix™ Bst Hot Start DNA Polymerase-V2 exhibits 5 '→ 3' DNA polymerase activity and strong strand displacement ability, lacks 3 '→ 5' exonuclease activity, making it suitable for DNA strand displacement reactions and LAMP amplification. AcuGenix™ Bst Hot Start DNA Polymerase-V2 is a hot start isothermal polymerase obtained by reversible modification technology based on Bst2.0. It can completely block enzyme activity at room temperature, establish reactions at room temperature, prevent non-specific amplification, and improve reaction efficiency. In addition, 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. 5×LAMP Premix Buffer 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
AcuGenix™ Bst Hot Start DNA Polymerase-V2 (8 U/μL)	1 μL	2 μL	0.32 U/μL
5×LAMP Premix Buffer Ⅱ	5 μL	10 μL	1×
RNase H Ⅱ (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 [3]	——	——	——
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. The 10×Probe Mix comprises : 1 μM probe primer prepared with TE Buffer.
3. In order to achieve better amplification results, appropriate templates are added, such as plasmids of 10 fg-1 ng, genomes of 1 ng-100 ng, and templates treated with appropriate amounts of DNA extraction solution.

Reaction Conditions

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

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.

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