

Product Description

AcuGenix™ Hot Start LAMP Dye Master Mix-V3 utilizes a dye-based method for isothermal amplification and can be used for the qualitative detection of DNA in samples (such as viruses). AcuGenix™ Bst Hot Start DNA Polymerase-V3 is derived from *Bacillus stearothermophilus* DNA Polymerase. Compared to the wild-type Bst DNA polymerase large fragment, this enzyme effectively enhances amplification speed and yield. AcuGenix™ Bst Hot Start DNA Polymerase-V3 possesses 5' → 3' DNA polymerase activity and strong strand displacement capability, but lacks 3' → 5' exonuclease activity, making it suitable for DNA strand displacement reactions and LAMP amplification. AcuGenix™ Bst Hot Start DNA Polymerase-V3 is a hot-start isothermal polymerase obtained by modifying Bst 3.0 with reversible modification technology. It can completely block enzyme activity at room temperature, allowing reactions to be set up at room temperature, preventing non-specific amplification, and improving reaction efficiency. Additionally, AcuGenix™ Bst Hot Start DNA Polymerase-V3 does not require a separate activation step.

Components

1. AcuGenix™ Bst Hot Start DNA Polymerase-V3 (8 U/μL)
2. TS-UNG II (1 U/μL)
3. 25mM dNTP Mix (064803)
4. 5×LAMP Lyophilization Buffer (064803)
5. 50×LAMP Fluorescent Dye II (064803)

Storage

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

Prepare Reaction Mix

Components	25 μL System	50 μL System	Final Concentration
5×LAMP Lyophilization Buffer (064803)	5 μL	10 μL	1×
AcuGenix™ Bst Hot Start DNA Polymerase-V3 (8 U/μl)	1.5 μL	3 μL	0.48 U/μl
TS-UNG II (1 U/μL)	1 μL	2 μL	0.04 U/μl
50×LAMP Fluorescent Dye II (064803) ¹	0.5 μL	1 μL	1×
10×Primer Mix ²	2.5 μL	5 μL	1×
Template DNA ³	---	---	---
ddH ₂ O	To 25 μL	To 50 μL	---

1. 50×LAMP Fluorescent Dye II (064803) can be substituted with other dyes. The optimal amount may vary depending on the dye used and needs to be determined experimentally.
2. 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.
3. Different types of DNA templates have different copy numbers of target genes. When necessary, gradient dilution can be performed to determine the optimal quantity of DNA template to be added.

Reaction Conditions

Incubate at a temperature range of 60-65 °C 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.