

Product Description

AcuGenix™ Lyo-Ready Hot Start LAMP Probe Master Mix-V2 is specifically developed for the lyophilization process, utilizing the probe method for qualitative and quantitative detection of DNA in samples (such as viruses). Bst2.0 HS in the product is derived from *Bacillus stearothermophilus* DNA Polymerase, exhibiting 5'→3' DNA polymerase activity and strong strand displacement capability, while lacking 3'→5' exonuclease activity. Compared to the wild-type Bst DNA polymerase large fragment, this enzyme effectively enhances amplification speed and yield. Obtained through reversible modification technology based on Bst 2.0, Bst2.0 HS can completely inhibit enzyme activity at room temperature, enabling reaction establishment at room temperature, preventing nonspecific amplification, and improving reaction efficiency. Additionally, Bst 2.0 HS does not require a separate activation step. This reagent utilizes an optimized formula of LAMP dye method lyophilization-specific Buffer, suitable for preparing full-component lyophilized products, such as lyophilized spheres and in-situ lyophilized powders.

Components

1. 10×LAMP Premix (DG) (Bst2.0 HS) (RNase H II)
2. 5×LAMP Lyophilization Buffer
3. 4×Lyophilization Protectant-9 (Optional)

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
10×LAMP Premix (DG) (Bst2.0 HS) (RNase H II)	2.5 µL	5 µL	1×
5×LAMP Lyophilization Buffer	5 µL	10 µL	1×
4×Lyophilization Protectant-9 ¹	6.25 µL	12.5 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. This system refers to a lyophilization system. When customers operate this system without lyophilization requirements, there is an option to add the 4×lyophilization protectant-9. In cases where lyophilized products are required, the 4×lyophilization protectant-9 must be added during the performance validation of the product at the liquid reagent stage to ensure consistency with the components and effects of the lyophilized system.
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

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

Prepare Reaction Mix for lyophilization

Components	25 µL System	50 µL System	Final Concentration
10×LAMP Premix (DG) (Bst2.0 HS) (RNase H II)	2.5 µL	5 µL	1×
5×LAMP Lyophilization Buffer	5 µL	10 µL	1×
4×Lyophilization Protectant-9	6.25 µL	12.5 µL	1×
10×Primer Mix	2.5 µL	5 µL	1×
ddH ₂ O	To 18-20 µL	—	—

*For other lyophilization preparation systems, please inquire separately.

Lyophilization Programe

Step	Temperature	Time	Condition	Pressure
Pre-freezing	4℃	30 min	Hold	1 atm
	-50℃	60 min	Cooling	
	-50℃	180 min	Hold	
First Drying	-30℃	60 min	Heating	Ultimate Vacuum
	-30℃	900 min	Hold	
Second Drying	25℃	60 min	Heating	Ultimate Vacuum
	25℃	300 min	Hold	

1. This freeze-drying process is an in-situ freeze-drying process for a 25 μ L system. If need to perform freeze-drying of freeze-dried beads or other in-situ systems, please inquire separately.
2. The above freeze-drying process is for reference only. Different product types and the use of different freeze dryers may result in different parameters, so adjustments can be made based on actual usage.
3. Different freeze-drying processes may be suitable for different batch sizes, and sufficient testing and verification must be conducted when used for large-scale production.

Protocol

1. Centrifuge the lyophilized sample immediately.
2. Add the nucleic acid template to the lyophilized sample and adjust the volume to 25 μ L with water.
3. Mix the sample thoroughly, centrifuge it, and load it onto the instrument.

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.