AcuGenix™ Lyo-Ready Hot Start Colorimetric LAMP Master Mix (Red-to-Yellow)-V2 V01

Product Description

AcuGenix™ Hot Start Colorimetric LAMP Master Mix (Red-to-Yellow)-V2 is specifically engineered for lyophilization processes and utilizes visual methods for the qualitative detection of RNA in specimens (e.g., viruses). Bst2.0 HS is derived from the DNA polymerase of *Bacillus stearothermophilus*. It exhibits 5'→3' DNA polymerase activity and strong strand displacement capability, while lacking 3'→5' exonuclease activity. Compared with the large fragments of wild-type Bst DNA polymerase, this enzyme significantly enhances the amplification speed and yield. It is obtained through reversible modification technology based on genetically engineered Bst 2.0 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. This reagent employs an optimized formulation of the LAMP visual assay buffer. It operates on the principle of pH reduction during the amplification reaction, enabling result interpretation through color changes. Before the reaction, the reagent appears purplish-red. After the reaction, positive wells turn yellow, while negative wells remain purplish-red. Additionally, Bst 2.0 HS does not require separate activation steps.

Components

1. 10×LAMP Premix Ⅱ (DG) (Bst2.0 HS)
2. 2×LAMP Premix Buffer (RY)
3. 4×AcuGenix™ Lyophilization Protectant-9 (optional)

Storage

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

Prepare Reaction Mix

Components	25 μL System	50 μL System	Final Concentration
10×LAMP Premix Ⅱ (DG) (Bst2.0 HS)	2.5 μL	5 μL	1×
2×LAMP Lyophilization Buffer (RY)	12.5 μL	25 μL	1×
4×AcuGenix™ Lyophilization Protectant-9 [1]	6.25 μL	12.5 μL	1×
10×Primer Mix [2]	2.5 μL	5 μL	1×
Template DNA [3]	——	——	——
ddH2O	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×AcuGenix™ Lyophilization Protectant-9. In cases where lyophilized products are required, the 4×AcuGenix™ 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

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.

Prepare Reaction Mix for lyophilization

Components	25 μL System	50 μL System	Final Concentration
10×LAMP Premix Ⅱ (DG) (Bst2.0 HS)	2.5 μL	5 μL	1×
2×LAMP Lyophilization Buffer (RY)	12.5 μL	25 μL	1×
4×AcuGenix™ 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_2O	To 25 μL	To 50 μL	——

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*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℃	210 min	Hold	
First Drying	-30℃	60 min	Heating	Ultimate Vacuum
	-30℃	720 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 μL 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 μL 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.