AcuGenix™ Lyo-Ready Hot Start RT-LAMP Probe Master Mix-V2 V01

Product Description

AcuGenix™ Lyo-Ready Hot Start RT-LAMP Probe Master Mix-V2 adopts the probe-based isothermal amplification method and can be used for qualitative detection of RNA in samples (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. Additionally, Bst 2.0 HS does not require separate activation steps. This premix does not contain glycerol components, thus it can be used for the development of freeze-dried products.

Components

1. 10×RT-LAMP Premix (DG) (Bst2.0 HS) (RNase H Ⅱ)
2. 5×RT-LAMP Lyophilization Buffer

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×RT-LAMP Premix (DG) (Bst2.0 HS) (RNase H Ⅱ)	2.5 μL	5 μL	1×
5×RT-LAMP Lyophilization Buffer	5 μL	10 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×
10×Probe Mix [3]	2.5 μL	5 μL	1×
Template RNA [4]	——	——	——
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. 10×Probe Mix : 1 μM probe primer in TE Buffer.
4. 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.

Reaction Conditions

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

Prepare Reaction Mix for lyophilization

Components	25 μL System	50 μL System	Final Concentration
10×RT-LAMP Premix (DG) (Bst2.0 HS) (RNase H Ⅱ)	2.5 μL	5 μL	1×
5×RT-LAMP Lyophilization Buffer	5 μL	10 μL	1×
4×AcuGenix™ Lyophilization Protectant-9	6.25 μL	12.5 μL	1×
10×Primer Mix	2.5 μL	5 μL	1×
ddH2O	To 25 μL	To 50 μL	——

*For other lyophilization preparation systems, please inquire separately.

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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 lyophilization procedure is specifically designed for the in-situ lyophilization of 25 μL systems. For the lyophilization of lyophilized beads or the in - situ lyophilization of other systems, separate inquiries should be made.
2. The aforementioned lyophilization procedure serves only as a reference. Variations in lyophilization parameters may occur due to different product types and lyophilizers, and these parameters can be adjusted according to actual circumstances during operation.
3. Different lyophilization procedures may be suitable for different lyophilization batches, and comprehensive testing and validation must be carried out 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 RT-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.