

Product Description

AcuGenix™ Lyo - Ready Hot Start Colorimetric RT - 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 (DG) 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 3.0 HS (DG) does not require separate activation steps.

Components

1. 10×RT-LAMP Premix II (DG) (Bst2.0 HS)
2. 2×LAMP Lyophilization Buffer (RY)
3. 4×Lyophilization Protectant (Sold separately)

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 II (DG) (Bst2.0 HS)	2.5 µL	5 µL	1×
2×LAMP Lyophilization Buffer (RY)	12.5 µL	25 µL	1×
4×Lyophilization Protectant ¹	6.25 µL	12.5 µL	1×
10×Primer Mix ²	2.5 µL	5 µL	1×
Template RNA 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. In cases where lyophilized products are required, the 4× lyophilization protectant 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 ddH₂O.

3. 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. This product functions as a pH - sensitive indicator reagent. High - concentration Tris - HCl buffer systems should be avoided in sample elution solutions. The use of strong acid or strong alkali nucleic acid release agents for sample processing should be avoided. If such reagents are unavoidably used, it is advisable to adjust the pH to neutral.

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 μ L System	50 μ L System	Final Concentration
10 $\times$ RT-LAMP Premix II (DG) (Bst2.0 HS)	2.5 μ L	5 μ L	1 $\times$
2 $\times$ LAMP Lyophilization Buffer (RY)	12.5 μ L	25 μ L	1 $\times$
4 $\times$ Lyophilization Protectant	6.25 μ L	12.5 μ L	1 $\times$
10 $\times$ Primer Mix	2.5 μ L	5 μ L	1 $\times$
ddH ₂ O	To 25 μ L	To 50 μ L	--

*For other lyophilization preparation systems, please inquire separately.

Lyophilization Progame

Step	Temperature	Time	Condition	Pressure
Pre-freezing	4 $^{\circ}$ C	30 min	Hold	1 atm
	-50 $^{\circ}$ C	60 min	Cooling	
	-50 $^{\circ}$ C	180 min	Hold	
First Drying	-30 $^{\circ}$ C	60 min	Heating	Ultimate Vacuum
	-30 $^{\circ}$ C	720 min	Hold	
Second Drying	25 $^{\circ}$ C	60 min	Heating	Ultimate Vacuum
	25 $^{\circ}$ C	300 min	Hold	

1. This lyophilization procedure is specifically designed for the in - situ lyophilization of 25 μ L 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 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.