AcuGenix™ Lyo-Ready Hot Start LAMP Dye Master Mix-V2

Product Description

LAMP (Loop-mediated isothermal amplification) is a nucleic acid amplification technique. This kit employs a dye-based isothermal amplification method, suitable for qualitative detection of DNA in samples such as viruses. Bst2.0 is derived from Bacillus stearothermophilus DNA Polymerase. Compared to the large fragment of wild-type Bst DNA polymerase, this enzyme effectively enhances amplification speed and yield. Bst2.0 is derived from the DNA polymerase of Bacillus stearothermophilus, exhibiting 5′→3′ DNA polymerase activity and strong strand displacement capability, while lacking 3′→5′ exonuclease activity. It can be used in DNA strand displacement reactions and LAMP amplification. Bst2.0 HS (DG) is a hot-start isothermal polymerase obtained through reversible modification technology based on Bst 2.0. It can completely inhibit enzyme activity at room temperature, allowing reactions to be established at room temperature, preventing nonspecific amplification and improving reaction efficiency. Additionally, Bst2.0 HS DNA (DG) polymerase does not require a separate activation step. This kit does not contain glycerol components, making it suitable for those with lyophilization conditions to develop lyophilized reagents.

Components

1. 10×LAMP Premix (DG) (Bst2.0 HS)
2. 5×LAMP Lyophilization Buffer
3. 50×LAMP Fluorescent Dye Ⅱ

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 (DG) (Bst2.0 HS)	2.5 μL	5 μL	1×
5×LAMP Lyophilization Buffer	5 μL	10 μL	1×
50×LAMP Fluorescent Dye Ⅱ [1]	0.5 μL	1 μL	1×
4×Lyophilization Protectant-9 [2]	6.25 μL	12.5 μL	1×
10×Primer Mix [3]	2.5 μL	5 μL	1×
Template [4]	——	——	——
ddH_2O	To 25 μL	To 50 μL	——

1. 50×LAMP Fluorescent Dye Ⅱ can be substituted with other dyes, and the dosage of different dyes may vary, requiring experimental adjustment.
2. 4×Lyophilization Protectant-9 can be substituted with other lyophilization protectants, and the dosage of different lyophilization protectants needs to be adjusted according to the experiment. When customers have no lyophilization requirements, 4×Lyophilization Protectant-9 can be added selectively; if there are lyophilization requirements, 4×Lyophilization Protectant-9 needs to be added during the product performance verification at the liquid reagent stage to ensure consistency with the system components and effects after lyophilization.
3. 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.
4. To achieve optimal amplification results, add appropriate templates, such as 10 fg-1 ng of plasmid, 1 ng-100 ng of genome, or an appropriate amount of template processed with DNA extraction buffer.

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.

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Prepare Reaction Mix for lyophilization

Components	25 μL System	50 μL System	Final Concentration
10×LAMP Premix (DG) (Bst2.0 HS)	2.5 μL	5 μL	1×
5×LAMP Lyophilization Buffer	5 μL	10 μL	1×
50×LAMP Fluorescent Dye Ⅱ	0.5 μL	1 μL	1×
4×Lyophilization Protectant	6.25 μL	12.5 μL	1×
10×Primer Mix	2.5 μL	5 μL	1×
ddH_2O	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℃	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.