AcuGenix™ Lyo-Ready Hot Start LAMP Probe Master Mix-V3

V01

Product Description

Loop-mediated isothermal amplification (LAMP) is a nucleic acid amplification technology. This kit adopts probe-based isothermal amplification and can be used for the qualitative detection of DNA from samples (e.g., viruses).AcuGenix™ Bst Hot Start DNA Polymerase-V3 is a thermosensitive isothermal amplification enzyme obtained via reversible modification technology based on genetically engineered AcuGenix™ Bst DNA Polymerase-V3 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. Furthermore, AcuGenix™ Bst Hot Start DNA Polymerase-V3 does not require separate activation steps. AcuGenix™ Bst DNA Polymerase-V3 is a next - generation Bst enzyme characterized by high sensitivity, excellent sample compatibility, and a certain tolerance to dUTP.

Components

1. 8 U/μL AcuGenix™ Bst Hot Start DNA Polymerase-V3 (Glycerol-Free)
2. 50 mU/μL AcuGenix™ RNase H Ⅱ (Glycerol-Free)
3. 5×LAMP Premix Buffer-T8ANT (Mg^{2+} free)
4. 35.71×$MgSO_4$
5. AcuGenix™ Lyophilization Protectant-9 (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
8 U/μL AcuGenix™ Bst Hot Start DNA Polymerase-V3 (Glycerol-Free)	1.5 μL	3 μL	0.48 U/μL
50 mU/μL AcuGenix™ RNase H Ⅱ (Glycerol-Free)	0.25 μL	0.5 μL	0.5 mU/μL
5×LAMP Premix Buffer-T8ANT (Mg^{2+} free)	5 μL	10 μL	1×
35.71×$MgSO_4$	0.7 μL	1.4 μL	1×
AcuGenix™ Lyophilization Protectant-9 (Sold separately)[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[4]	——	——	——
ddH_2O	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 AcuGenix™ Lyophilization Protectant-9 (Sold separately). In cases where lyophilized products are required, the AcuGenix™ Lyophilization Protectant-9 (Sold separately) 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. 10×Primer Mix: 16 μM FIP, 16 μM BIP, 2 μM F3, 2 μM B3, 4 μM LoopF and 4 μM LoopB in TE。
3. 10×Probe Mix : 1 μM probe primer in TE。
4. For optimal amplification, add an appropriate amount of template: plasmid DNA 10 fg-1 ng, genomic DNA 1 ng-100 ng, or appropriately prepared DNA extract.

Reaction Conditions

Incubate at a temperature range of 60 - 65°C 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 Conc.
8 U/μL AcuGenix™ Bst Hot Start DNA Polymerase-V3 (Glycerol-Free)	1.5 μL	3 μL	0.48 U/μL
50 mU/μL AcuGenix™ RNase H Ⅱ (Glycerol-Free)	0.25 μL	0.5 μL	0.5 mU/μL
5×LAMP Premix Buffer-T8ANT (Mg^{2+} free)	5 μL	10 μL	1×
35.71×$MgSO_4$	0.7 μL	1.4 μL	1×
AcuGenix™ Lyophilization Protectant-9 (Sold separately)	6.25 μL	12.5 μL	1×
10×Primer Mix	2.5 μL	5 μL	1×
10×Probe Mix	2.5 μL	5 μL	1×
ddH_2O	To 18.5 μL	To 30 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 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 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.