EZassay

RT-LAMP Kit (UDG enzyme based contamination control)-Lyophilized

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Catalog Code: RT-LAM-LYO-UDG-1
RT-LAM-LYO-UDG-10

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Product Introduction

The RT-LAMP kit is based on a thermostable reverse transcriptase and an optimized Bst 2.0 DNA polymerase system combined with aptamer-mediated hot-start technology, enabling rapid reverse transcription and amplification of target RNA under isothermal conditions. Reverse transcription and amplification are performed sequentially in a single reaction system, offering simple operation and high efficiency.

At low temperatures, the aptamer specifically binds to the enzyme and inhibits its activity. Upon reaching the reaction temperature, the aptamer undergoes a conformational change and dissociates from the enzyme, thereby restoring enzyme activity and initiating amplification. This mechanism effectively reduces non-specific amplification during reaction setup and significantly improves assay specificity and reproducibility.

The kit provides a complete reaction system along with a positive control, facilitating rapid method setup and reliable quality control. It is suitable for molecular diagnostics and POCT application development.

In addition, the incorporation of dUTP and heat-labile UDG effectively minimizes the risk of carryover contamination. The heat-labile UDG is fully inactivated at temperatures above 50 °C, ensuring no interference with the amplification reaction.

Materials supplied

Component	RT-LAM-LYO-UDG-1	RT-LAM-LYO-UDG-10
RT-LAMP Enzyme Mix (25X) (lyophilized)*	96T	96T*10
LAMP Reaction Buffer (2X) (dUTP included)	0.7mL*2	0.7mL*20
UDG enzyme (1U/μL) (lyophilized)*	1vial	1vial*10
Positive Control	80μl	800μl
PCR-grade water	1mL	1mL*2

* Add PCR-grade water according to the label on the tube before use.
Note: Before opening the tube, briefly centrifuge at 3000-4000 rpm for 1 minute to collect the reagents at the bottom of the tube.

Storage

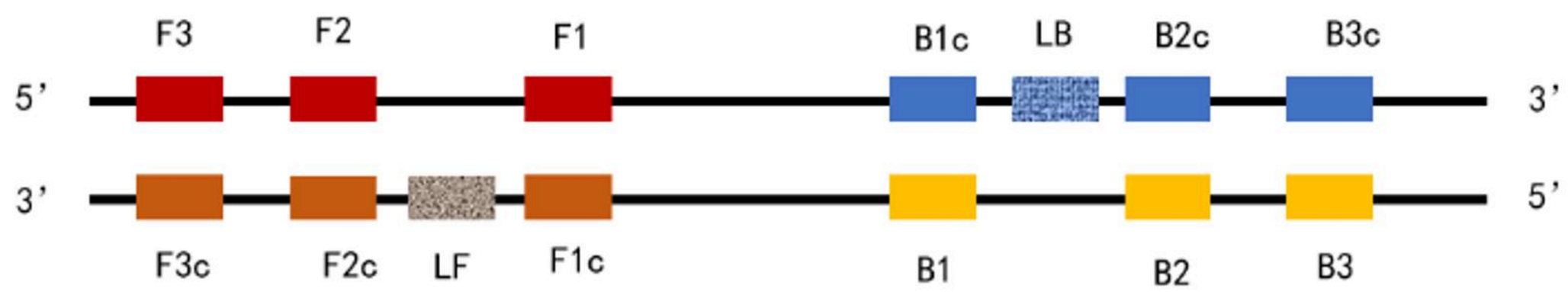
Store at -20 °C. After rehydration, aliquot as needed. Avoid repeated freeze-thaw cycles.

Heat Inactivation

85°C for 10 minutes.

Required materials

i. Thermal cycler or heat block
ii. Template RNA
iii. Primers (Recommend LAMP primer online software: https://lamp.neb.com/#!/)



- F1c: Complementary sequence of F1
- FIP: Forward Inner Primer (F1c + F2)
- LF: Forward loop primer
- B1c: Complementary sequence of B1
- BIP: Backward Inner Primer (B1c + B2)
- LB: Backward loop primer

Preparation of reagents

1、 Prepare reactions as described in the table below. Suggest to set up the reaction on ice.

Component	Volume	Working concentration
LAMP Reaction Buffer (2X) (dUTP included)	12.5μl	1X
RT-LAMP Enzyme Mix (25X)	1μl	1X
UDG enzyme (1U/μL)	1μl	1U total

Primers (10X)	2.5µl	1.6 µM FIP/BIP, 0.4 µM LF/LB, 0.2 µM F3/B3,
Template RNA**	1µl	>10 copies or more
PCR-grade water	Up to 25µL	-

* Example of Preparing 10X Primers Mix:

i. Primer stock concentrations: Dilute FIP and BIP primers to 80 µM; dilute LF, LB, F3, and B3 primers to 20 µM.

ii. Prepare the Mix:

¢ FIP, BIP, LF, LB primers: 2µL each

¢ F3, B3 primers: 1µL each

¢ Total volume 10µL.

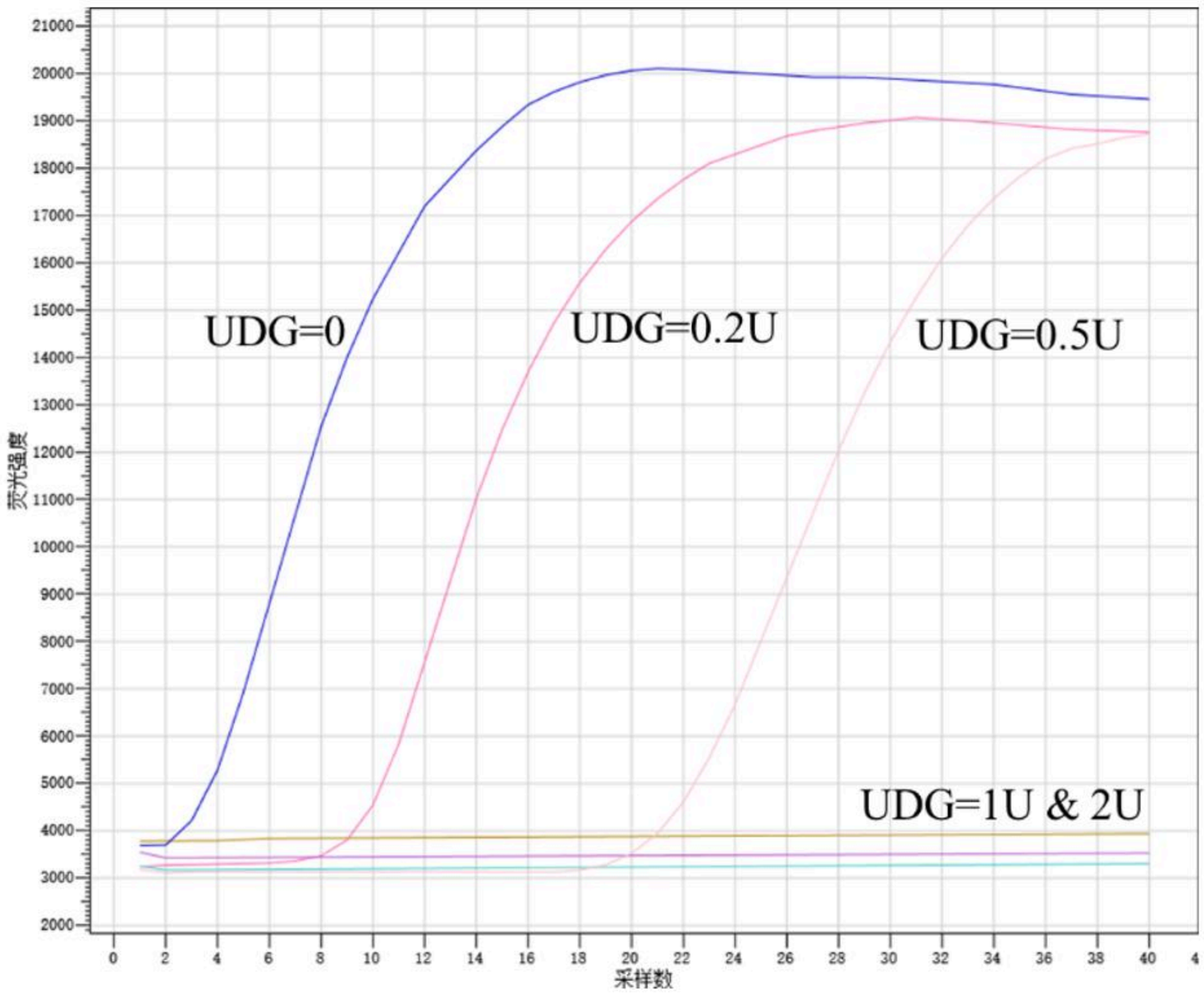
(Note: Primers may be diluted to other concentrations. In such cases, the volume added should be calculated accordingly to meet the final concentration requirements in the reaction system.)

**For positive control group, add 8µl Positive Control that provided in the kit. Template and primers are already mixed as positive control.

2、 Incubate the reaction at 65°C for 30 to 60 minutes.

3、 The amplification products can then be analyzed by electrophoresis on a 2% agarose gel. If required, a fluorescent dye may be added to the reaction system, and fluorescence signals can be measured every minute using a real-time PCR instrument.

Example result of UDG enzyme degradation of LAMP amplification products



Amplification products containing dUTP were used as templates. After a 10-fold dilution, 2 μL of the diluted sample was subjected to LAMP amplification. Strong fluorescent signals were observed in the reaction system without UDG, whereas the signals disappeared upon addition of UDG. This result demonstrates that UDG can effectively degrade dUTP-containing amplification products, thereby preventing false positives caused by carryover contamination.

Attention

- LAMP Reaction Buffer is formulated with a mixture of dTTP and dUTP. This ensures both efficient isothermal amplification as well as the incorporation of dU into the reaction products.
- Designate and use distinct areas for sample preparation, reaction setup and analysis to avoid carry-over contamination.
- Running a negative control is strongly recommended to ensure amplification specificity.
- If PCR thermal cycler is used, turn off the lid heating function or set it to 70 °C.
- For projects prone to non-specific signals, it is recommended to use AapCas12b (Cat. No. CAS-12P-010), BrCas12b (Cat. No. CAS-12R-010), or RNaseH2 (Cat. No. RH2-250) to enhance specificity.

How to choose the LAMP products

	Product name	Catalog number	Brief introduction
1	• LAMP Kit • RT-LAMP Kit	• LAM-LYO-1 • RT-LAM-LYO-1	Basic (RT-)LAMP amplification for DNA/RNA detection; compatible with fluorescence, colorimetric, or lateral flow readout.
2	• Colorimetric LAMP kit • Colorimetric RT-LAMP kit	• LAM-LYO-RED-1 • RT-LAM-LYO-RED-1	Visual (RT-)LAMP detection by simple color change; no instrument required.
3	• LAMP Fluorescent Kit • RT-LAMP Fluorescent Kit	• LAM-LYO-EGR-1 • RT-LAM-LYO-EGR-1	Fluorescent (RT-)LAMP detection with EvaGreen; compatible with fluorescence readers.
4	• One-Step LAMP-CRISPR DNA Fluorescence Detection Kit • One-Step RT-LAMP-CRISPR DNA Fluorescence Detection Kit	• LAM-CAS-LYO-01 • RT-LAM-CAS-LYO-01	One-step (RT-)LAMP-CRISPR detection with enhanced specificity; fluorescence or lateral flow readout.
5	• RNase H2 Probe-Based LAMP Isothermal Amplification Kit • RNase H2 Probe-Based RT-LAMP Isothermal Amplification Kit	• LAM-PB-LYO-1 • RT-LAM-PB-LYO-1	Probe-based (RT-)LAMP with enhanced specificity; suitable for SNP detection.

How to choose the LAMP-related enzymes

LAMP related enzymes	Catalog Number	Brief introduction
Bst DNA Polymerase 2.0	BST2-1600	DNA polymerase with strong strand displacement activity, suitable for LAMP amplification reactions. High sensitivity and specificity.
Thermostable reverse transcriptase (for LAMP)	RT-TS-LM-2000	High-temperature resistant reverse transcriptase. The ideal partner for LAMP and PCR!
Thermostable RNaseH2/RNaseH II	RH2-250	RNase H2 is a specialized enzyme that degrades RNA in RNA/DNA hybrids
Uracil DNA Glycosylase (UDG/UNG), heat-labile	UG-HL-100	UDG acts on single- or double-stranded DNA containing dUTP and is inactive against RNA. It is sensitive to high temperature, and can irreversibly inactivate the enzyme above 50° C, and is suitable for LAMP, RT-LAMP, q-PCR and RT-qPCR.

Professional supplier of point-of-care test products

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