Fluorescent LAMP/RT-LAMP Kit

RP711

Version 23.1

Product Description

Fluorescent LAMP/RT-LAMP Kit is designed to provide a simple one-step solution for loop-mediated isothermal amplification of DNA or RNA targets. The 2 × Fluorescent LAMP/RT-LAMP Master Mix in this kit contains optimized buffer solution, dNTP, Bst II Pro DNA polymerase and RTv reverse transcriptase for fast and efficient LAMP/RT-LAMP detection. Bst II Pro DNA Polymerase and RTv Reverse Transcriptase use the updated hot start technology, which has excellent specificity and supports the preparation of reaction system at room temperature. This kit provides fluorescent dyes to realize real-time fluorescence detection of LAMP/RT-LAMP.

Components

Components	RP711-01 (100 rxns)	RP711-02 (500 rxns)
2 × Fluorescent LAMP/RT-LAMP Master Mix	1.25 ml	5 × 1.25 ml
50 × LAMP Fluorescent Dye[a,b]	50 µl	250 µl

a. It is stored in DMSO.

b. Adjust the final concentration of LAMP Fluorescent Dye according to different instruments. It is recommended that the final concentration of ABI QuantStudio 3, ABI QuantStudio 5, ABI StepOnePlus, Roche LightCycler 96, Bio-rad CFX96 Touch and BIOER QuantGene 9600 is 1 ×. For other types of instruments, it is recommended to use the final concentration of dye at 0.1 - 1 ×.

Storage

Store at -30 ~ -15°C and transport at ≤0°C.

Applications

It is applicable for isothermal amplification reactions of LAMP/RT-LAMP.

Self-prepared Materials

Reagents: FIP/BIP Primers, F3/B3 Primers, LoopF/LoopB Primers, Nuclease-free ddH_2O.

Instruments: qPCR instrument.

Notes

For research use only. Not for use in diagnostic procedures.

Experiment Process

Take Fluorescent LAMP/RT-LAMP as an example:

1. Thaw Fluorescent LAMP/RT-LAMP Kit to room temperature completely. Vortex for 10 sec to mix thoroughly before use, then centrifuge briefly to the bottom of the tube.
2. Follow the table below to prepare the reaction system. The template should be added in the last step.

Components	Volume	Final Concentration
2 × Fluorescent LAMP/RT-LAMP Master Mix	12.5 μl	1 ×
FIP/BIP Primers (100 μM)	0.4 μl each	1.6 μM each
F3/B3 Primers (100 μM)	0.05 μl each	0.2 μM each
LoopF/LoopB Primers (100 μM)	0.2 μl each	0.8 μM each
50 × LAMP Fluorescent Dye	0.5 μl	1 ×
DNA/RNA Template	1.0 - 5.0 μl	
Nuclease-free ddH_2O	up to 25 μl	

▲If the amount of primers is small, it is recommended to premix the primers first.

▲It is recommended to prepare reagents and templates in different areas to avoid contamination.

3. Vortex to mix thoroughly, then centrifuge briefly to the bottom of the tube.

▲Make sure there are no air bubbles in the reaction system.

4. Add template DNA/RNA. The final volume of the reaction system should be 25 μl.

▲It is recommended to add the template last to ensure the reliability of results.

5. Vortex to mix thoroughly, then centrifuge briefly to the bottom of the tube.
6. Perform LAMP/RT-LAMP reaction on qPCR instrument according to the following program.

Stage 1	Cycling Reaction	Reps: 30 - 60[a]	60 ~ 65℃[b]	60 sec
Stage 2	Melting Curve[c]	Rep: 1	95℃	15 sec
			60℃	60 sec
			95℃	1 sec

a. According to different primers and templates, the number of reaction cycles can be optimized between 30 - 60 cycles.

b. According to different primers and templates, the optimal reaction temperature can be optimized between 60 ~ 65°C.

c. Different types of instruments have different acquisition procedures of the melting curve. It is recommended to use the default collection procedure of the instrument.

FAQ & Troubleshooting

◇ How to design and screen primers for loop-mediated isothermal amplification?

Please refer to http://primerexplorer.jp/e/ for primer design. Version 5 is recommended.

Log in to http://primerexplorer.jp/lampv5e/index.html to download the manual.

For preliminary screening, please refer to the manual. The optimal primer need to be verified by experiments.

◇ Is a separate step of reverse transcription required to detect RNA targets?

No separate step of reverse transcription is required. RTv reverse transcriptase can efficiently synthesize cDNA at 60 ~ 65°C.