LAMP Fluorescent Dye

RP001

Version 23.1

Product Description

LAMP Fluorescent Dye is an intercalating dye with the strong fluorescent signal and little inhibition of the reaction. It is applicable for real-time fluorescent detection of LAMP reactions. This product is a 50 × solution with DMSO as the solvent, compatible with a variety of fluorescent quantitative PCR instruments. It is recommended that the final concentration of the dye be 0.1 - 1 ×, and please select the SYBR/FAM detection channel when using it.

Components

Components	RP001-01 (100 μl)
50 × LAMP Fluorescent Dye[a, b]	100 μl

a. Store in DMSO.

b. Adjust the final dye concentration according to different instruments. It is recommended that the final dye concentration of ABI QuantStudio 3, ABI QuantStudio 5, ABI StepOnePlus, Roche LightCycler96, Bio-rad CFX96 Touch, BIOER QuantGene 9600, and Tianlong GENtier 96R be 1 ×. For other models of qPCR instruments, the final dye concentration of 0.1 - 1 × is recommended.

Storage

Store at -30 ~ -15°C and protect from light. Transport at ≤0°C.

▲ Avoid repeated freezing and thawing.

Applications

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

Self-prepared Materials

Reagents: Bst DNA Polymerase Large Fragment, Buffer, $MgSO_4$, dNTP Mix, FIP/BIP Primers, F3/B3 Primers, LoopF/LoopB Primers and Nuclease-free ddH_2O.

Machine: qPCR instruments.

Notes

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

Experiment Process

Take LAMP isothermal amplification as an example

1. Take out the 50 × LAMP Fluorescent Dye and equilibrate to room temperature to ensure that the dye is completely thawed. Vortex 10 sec to mix before use, then centrifuge it briefly to the bottom of the tube.
2. Prepare the reaction system according to the table below, and add the template last.

Components	Volume	Final Concentration
10 × IsothermalAmp Buffer	2.5 μl	1 ×
$MgSO_4$ (100 mM)	1.5 μl	6 mM (total 8 mM)
dNTP Mix (10 mM each)	3.5 μ	1.4 mM each
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
Bst DNA Polymerase Large Fragment (8 U/μl)	1.0 μl	0.32 U/μl
50 × LAMP Fluorescent Dye	0.5 μl	1 ×
DNA Template	1.0 - 5.0 μl	
Nuclease-free ddH_2O	up to 25 μl	

▲ According to different experiments, the concentration of Mg^{2+} can be adjusted in the range of 6 - 10 mM.

▲ Due to the low amount of primers, 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 and centrifuge briefly to collect to the bottom of the tube.

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

4. Add the corresponding volume of DNA templates to make the total volume to 25 μl.

▲ Due to the reaction is relatively fast, it is recommended to add the template last for ensuring stable and reliable results.

5. Vortex to mix and centrifuge briefly to collect to the bottom of the tube.
6. Perform the LAMP reaction on the qPCR instrument under the following Reaction program.

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

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

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

c. Different instruments have different melting curve acquisition procedures. Just use the default melting curve acquisition program.