RT-LAMP Fluorescent Master Mix

Cat.No.:HYB413

Product Description

This product contains reaction buffer, RT-enzymes mix of Bst DNA polymerase and heat-resistant reverse transcriptase, lyophilized protectant and fluorescent dye components. The reaction buffer contains Mg^{2+}, dNTP and other necessary components for amplification, users can directly mix the reaction buffer, RT enzymes mix, fluorescent dye, primer, and then add the template. If necessary, users can add the lyophilized protectant to prepare lyophilizable system.

Components

Components	96 T	960 T	9600 T
Loop-mediated Amplification Buffer	1.2 mL	4 mL×3	12 mL×10
RT-Enzymes Mix	245 μL	2.45 mL	2.45 mL×10
Lyophilized protectant	0.96 mL×2	9.6 mL×2	9.6 mL×20
25× Dye	192 μL	0.96 mL×2	0.96 mL×20

Storage

Store at -25～-15℃, valid for 12 months.

Instructions

1. Thaw the reaction buffer to be used at room temperature. Vortex briefly or invert tubes several times to mix thoroughly, then centrifuge to collect the liquid to the bottom of the tube.
2. Preparation of reaction system: this reagent can be prepared in two reactionb systems, liquid reaction mix and Freeze- drying system mix.

1) Prepare liquid reaction mix

Components	Volume
Loop-mediated Amplification Buffer	12.5 μL
25× Dye	2 μL
RT-Enzymes Mix	2.55 μL
10×Primer Mix*	5 μL
Template DNA/RNA**	x μL
Nuclease-free Water	Up to 50 μ L

2) Freeze-drying system mix

① Prepare freeze-drying mix

Components	Volume
Loop-mediated Amplification Buffer	12.5 μL
Lyophilized protectant	20 μL
25× Dye	2 μL
RT-Enzymes Mix	2.55 μL
Nuclease-free Water	Up to 50 μ L

② Freeze dry

The prepared Mix was freeze-dried in a 50μL system.

③ Prepare reaction mix

Components	**Volume**
Freeze-dried mix	1 piece
10×Primer Mix*	5 μ L
Template DNA/ RNA**	x μ L
Nuclease-free Water	Up to 50 μ L

*10 × Primer Mix Concentration: 16 μMFIP/BIP, 2 μM F3/B3, 4 μM Loop F/B;

**DEPC (water soluble) is recommended for nucleic acid templ.

3. Reaction program

Set the parameters on the PCR instrument (such as ABI7500) as follows:

Temperature	**Time**	**Cycles**
65℃	60s *Collect fluorescent signal	30

Notes

1. Salt may appear in the bottom of the buffer tube, vortex briefly or invert tubes several times to mix thoroughly at room temperature.
2. The reaction temperature can be optimized between 62°C and 68°C according to the primer conditions.
3. The experiment shall be conducted in a standardized manner, including the preparation of reaction system, sample treatment and sample addition.

4. It is suggested to prepare reaction system in the ultra-clean table and add templates in the fume hood of other rooms to avoid false.