

Isothermal nucleic acid amplification reagent

LAMP MASTER Series

I . Introduction

2×LAMP MASTER is an optimized master mix to simplify the preparation for isothermal amplification assays. This product contains Bst DNA polymerase, inorganic pyrophosphatase, Mg²⁺, dNTPs, and optimized reaction buffer. Three types of products in this product can be selected depending on the detection method.

Notes

- This product is for research use only and is not for pharmaceutical use.
- LAMP (Loop-Mediated Isothermal Amplification) is a rapid, simple and specific gene amplification method developed by Eiken Chemical Co., Ltd

II . Kit Components

LAMP MASTER for Turbidity

(Code No.311-08961 / 300 reactions)

Reagent	Amount	Storage	Detection
2 x LAMP MASTER	625 µL × 6	-20°C	Turbidity

LAMP MASTER for Fluorescence

(Code No.317-08941 / 1set)

Reagent	Amount	Storage	Detection
2 x LAMP MASTER	625 µL × 6	-20°C	Fluorescence
10 x Intercalation Mix	750 µL × 1	-20°C(*)	

*Protect from light

LAMP MASTER for Turbidity (Visible Dye)

(Code No. 314-08951 / 1set)

Reagent	Amount	Storage	Detection
2 x LAMP MASTER	625 µL × 6	-20°C	Visual
25 x Visible Dye	300 µL × 1	-20°C(*)	

*Protect from light

III. Protocol

Preparation of reagents

- ① Prepare the Nucleic acid template with suitable extraction method for each sample.

Important Notes To avoid contamination of the test environment, prepare the nucleic acid template separately from the area where LAMP reaction will be performed.

- ② Prepare the 10 x LAMP Primer Mix by combining 4 or 6 different primers designed to recognize regions on the target gene.

Example 10 × LAMP Primer Mix : 16 µM FIP, 16 µM BIP, 2 µM F3 Primer, 2 µM B3 Primer, 8 µM Loop Primer F, 8 µM Loop Primer B, 10 mM Tris-HCl (pH 8.0), 1 mM DTT

- ③ Completely thaw the reagents required for the reaction, such as 2 x LAMP MASTER, Nucleic acid template, Primer etc. at room temperature. Mix each reagent 3 times for 1 second using a vortex mixer to make it homogeneous, then spin down and keep it on ice.

* The 10 × Intercalation Mix and 25 × Visible Dye should be thawed under light-shielded conditions such as in aluminum bag or wrapped in aluminum foil.

Preparation of reaction solution

- ④ Refer to the reaction examples, add the required amounts of reagents for the reaction except for the nucleic acid template into 1.5 ml or 2.0 ml tubes and then mix 3 times for 1 second using a vortex mixer and spin down and keep it on ice as a master mix.

Reference When using AMV Reverse Transcriptase in the RT-LAMP, add 0.2 units per 25 µl reaction.

- ⑤ Dispense the master mix for one reaction into the recommended tubes for the detection device used. Add ~5 µl of nucleic acid template or control(*) to make a total reaction volume to 25 µl.

* First, add negative control (such as sterile distilled water), and finally positive control. Cap it after adding. At this time, mix well

by pipetting or tapping with the cap closed, and then spin down.

Be careful not to make bubbles when mixing.

- ⑥ Place the tube in the detection device and perform the LAMP reaction at 60-68°C for 30-60 minutes.

*The optimal temperature depends on the designed primer, so evaluate the conditions in advance before deciding on the reaction temperature.

*For reaction time, refer to the reaction examples below.

Reaction example.

LAMP MASTER for Turbidity

2 × LAMP MASTER	12.5 µl
10 × LAMP Primer Mix	2.5 µl
Nucleic acid template	~5 µl
Sterile distilled water	up to 25 µl
↓	
60~68°C, 60 min. (LAMP reaction)	

<Turbidity detection>

Magnesium pyrophosphate, a reaction by-product accumulates through DNA amplification and cause the reaction solution to become turbid. By measuring this turbidity with a turbidimeter, target gene can be detected.

Reaction example.

LAMP MASTER for Fluorescence

2 × LAMP MASTER	12.5 µl
10 × Intercalation Mix	2.5 µl
10 × LAMP Primer Mix	2.5 µl
Nucleic acid template	~5 µl
Sterile distilled water	up to 25 µl
↓	
60~68°C, 30 min. (LAMP reaction)	
↓	
Melting curve analysis	

<Fluorescence detection>

An intercalator dye contained in 10×Intercalation Mix generates fluorescence through binding to double-stranded DNA and enables detection of DNA amplification by using a fluorescence detection device or a real-time PCR instrument capable of detecting SYBR™ Green I or ResoLight Dye.

Notes 10×Intercalation Mix is a fluorescence detection reagent and cannot be used for turbidity measuring device.

Reaction example.

LAMP MASTER for Turbidity (Visible Dye)

2 × LAMP MASTER	12.5 µl
25 × Visible Dye	1.0 µl
10 × LAMP Primer Mix	2.5 µl
Nucleic acid template	~5 µl
Sterile distilled water	up to 25 µl
↓	
60~68°C, 40 min. (LAMP reaction)	
80°C, 5 min. (Inactivation reaction)	
↓	
Visual detection	

<Visual detection>

Under visible light, pale red color appears for negative reaction and clear yellow green color appears for positive reaction. More accurate determination is possible under UV irradiation.

Important point In the case of visual detection, be sure to react the negative and positive control at the same test, and confirm whether the color development appears in the control solutions before determining the sample. (It is possible to extend the reaction time up to 60 minutes, but in this case, use a negative control to confirm the absence of non-specific reaction.)

Notes Leaving the LAMP reaction solution for extended periods may cause fluorescence to develop or quench, regardless of the reaction's progress, leading to erroneous results. Therefore, in visual detection, results should be determined immediately after the reaction has stopped. Furthermore, do not use TE containing chelate metals such as EDTA when preparing the reaction solutions.