WarmStart LAMP Kit (DNA & RNA) Protocol (E1700)

Materials Required but not Supplied

WarmStart® LAMP Kit（DNA & RNA）

- LAMPプライマー（NEB LAMP Primer Design Toolの使用を推奨する）
- ターゲット核酸サンプル
- 分子生物学グレードのH_2O
- ヒートブロック、ウォーターバス、リアルタイム濁度計またはサーマルサイクラー（必要であればリアルタイム蛍光測定を含む）および機器に適した反応容器

Overview

Reaction Setup: *For simplicity in setting up reactions, we recommend making stocks of the LAMP primers at a usable concentration. For example, we suggest a 10X Primer Mix containing all 6 LAMP primers.*

A 10X LAMP Primer Mix contains:

PRIMER	10X CONCENTRATION (STOCK)	1X CONCENTRATION (FINAL)
FIP	16 µM	1.6 µM
BIP	16 µM	1.6 µM
F3	2 µM	0.2 µM
B3	2 µM	0.2 µM
LOOP F	4 µM	0.4 µM
LOOP B	4 µM	0.4 µM

Prepare primer stocks in nuclease-free water and store at –20°C for up to 2 years.

1. Thaw all components to be used at room temperature and place on ice. Vortex briefly to mix and centrifuge to collect material.

2. Prepare reaction mix as described below. Volumes are listed for a 25 µl LAMP reaction, but other volumes (10, 20, 50 µl etc.) are all effective; if desired, adjust volumes accordingly. A 1 µl target DNA volume is shown; if higher sample volumes are needed, adjust volume of H_2O. For non-template reactions add equivalent volume of H_2O or sample storage buffer.

	DNA TARGET DETECTION	RNA TARGET DETECTION	NO-TEMPLATE CONTROL (NTC)
WarmStart LAMP 2X Master Mix	12.5 µl	12.5 µl	12.5 µl
Fluorescent dye (50X)	0.5 µl	0.5 µl	0.5 µl

LAMP Primer Mix (10X)	2.5 µl	2.5 µl	2.5 µl
Target DNA	1 µl	–	–
Target RNA	–	1 µl	–
dH_2O	8.5 µl	8.5 µl	9.5 µl
Total Volume	25 µl	25 µl	25 µl

3. Vortex reaction mix and centrifuge to collect material.

4. Pipet 24 µl per reaction into desired reaction vessels and add sample. Mix by vortexing and centrifuge to collect, or by pipetting if using a plate or other vessel.

5. Seal reaction vessel.

6. Incubate at 65°C for 30 minutes. Time can be extended as necessary for very low copy targets, challenging sample types, or reactions known to be produce slower amplification times.

7. If reaction products will be manipulated or analyzed after LAMP is complete, *Bst* 2.0 and RTx can be inactivated by heating at > 80°C for 5 minutes.