Colorimetric RapidLAMP 2X Master Mix with UDG (# RL1000)
Typical LAMP Protocol

Reaction Setup: For simplicity in setting up reactions, we recommend making stocks of the LAMP primers at a usable concentration. For example, we suggest 10X Primer Mix (100 reactions) containing all 6 LAMP primers.

10X (100 reactions) LAMP Primer Mix contains:

Primer	Primer Concentration	Primer Addition	Final Concentration
FIP	100 µM	32 µl	1.6 µM
BIP	100 µM	32 µl	1.6 µM
F3	100 µM	4 µl	0.2 µM
B3	100 µM	4 µl	0.2 µM
Loop F	100 µM	8 µl	0.4 µM
Loop B	100 µM	8 µl	0.4 µM
dH_2O		112 µl	

Note: Make primer stock in molecular biology grade H_2O rather than TE in order to avoid carryover of additional buffer to the LAMP reaction. Prepare primer stocks in nuclease free water and store at –20°C for up to 2 years.

1. Thaw the Colorimetric RapidLAMP 2X Master Mix and the 10X Primer Mix to be used at room temperature and place on ice. Salt may appear in the bottom of the tube so vortex briefly or invert tubes several times to mix thoroughly. Centrifuge to collect material and place on ice.

2. Prepare reaction mix as described below using Colorimetric RapidLAMP Master Mix, LAMP primers and nuclease free water. Volumes listed per 20 µl LAMP reaction, but other volumes (10, 25, 50 µl etc.) are all effective if desired, just adjust volumes accordingly. Sample is assumed here to be 8 µl, but for lower sample volumes add as needed and increase volume of H_2O to compensate. For non-template reactions add equivalent volume of H_2O or sample storage buffer.

	DNA Target	**RNA Target**	**NO Template Control (NTC)**
Colorimetric RapidLAMP 2X Master Mix with UDG	10 µl	10 µl	10 µl
LAMP Primer Mix (10X)	2 µl	2 µl	2 µl
Target DNA	8 µl	-	-
Target RNA	-	8 µl	-
dH_2O	-	-	8 µl
Total Volume	**20 µl**	**20 µl**	**20 µl**

3. Gently vortex the reaction mix and briefly centrifuge to collect material.

4. Pipet 12 µl per reaction into desired reaction vessels and add 8 µl of sample. Mix by vortexing or by pipetting if using a plate or similar vessel, briefly centrifuge to collect if necessary. Check that reaction solutions have a purple color, which indicates initial high Mg^{2+} required for successful LAMP reaction.

5. Seal reaction vessels. For maximum sensitivity, transfer reactions from room temperature to a preheated block as it warms to 65°C with 100°C heated lid. Reactions should not be allowed to sit on the block as it warms to 65°C, as this will result in competing DNA synthesis by WarmStart RTx and *Bst* 3.0 and dU-containing amplicon destruction by Thermolabile UDG.

6. Incubate at 65°C for 40 minutes.

7. Remove tubes or vessels from incubation and examine by eye. Positive reactions will have turned blue while negative controls should remain purple. If color change is not robust, return reactions to 65°C for an additional 10 minutes. Reactions can be examined earlier if desired, and high copy or input reactions can exhibit full color change in as little as 30 minutes.

8. The result can be photographed and quantified using a mobile application or the PKT RGB Reader™ to the colorimetric results (RGB score).

Note: DO NOT open the reaction tubes as they contain a very high amount of amplified DNA and may cause contamination of the lab and equipment.

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