Typical LAMP Protocol (M0275)

Overview

Incubate the following reaction at 65°C for 30–60 minutes.

Component	25 µl Reaction	Final Conc
10X ThermoPol Buffer	2.5 µl	1X (contains 2 mM $MgSO_4$)
$MgSO_4$ (100 mM)	1.5 µl	6 mM (8 mM total)
dNTP Mix (10 mM)	3.5 µl	1.4 mM each
FIP/BIP Primers (25X)	1 µl	1.6 µM
F3/B3 Primers (25X)	1 µl	0.2 µM
LoopF/B Primers (25X)	1 µl	0.4 µM
Bst DNA Polymerase, Large Fragment (8,000 U/ml)	1 µl	320 U/ml
DNA Sample	variable	> 10 copies or more
Nuclease-free Water	to 25 µl	
Total Reaction Volume		25 µl

General Guidelines:

1. A LAMP Primer Mix can be prepared with all 4 or 6 (with Loop) primers. A 25X Primer Mix should contain: 40 µM FIP, 40 µM BIP, 5 µM F3, 5 µM B3, 10 µM LoopF, 10 µM LoopB in TE or water.
2. Reactions should be setup on ice. If room temperature setup is desired, use *Bst* 2.0 WarmStart® DNA Polymerase (NEB #M0538).
3. If analyzing via agarose gel electrophoresis or other method requiring opening LAMP reaction vessels, setup secondary analysis area and equipment to avoid contamination.
4. Running a no-template control is strongly recommended to ensure amplification specificity.
5. If optimization is desired, try titrating Mg^{2+} (4–10 mM final) or *Bst* DNA Polymerase, Large Fragment (0.04–0.32 U/µl), or changing reaction temperature (50–68°C).