QUICK PROTOCOL

LavaLAMP™ DNA Master Mix

(CAT. NO. 30066-1, 30067-1)

1. Thaw all kit components on ice and set up reactions on ice.
2. Mix each component thoroughly before use by vortexing for 3 - 10 sec. Spin down contents at 4°C.
3. Prepare reaction mixes in the order listed below in Table 1. When possible, make enough of the reaction mixes for all your reactions at one time.

   Note: The reaction conditions recommended in Table 1 assume you will use the Green Fluorescent Dye available with Cat. No. 30067-1 and 1 µL of Target DNA Sample in step 6. If you are not using the Green Fluorescent Dye and/or are using a different volume of Target DNA Sample, adjust the amount of nuclease-free H_2O accordingly.

Table 1. Positive Control, No Target Control and Experimental Reaction Setup

	Positive Control	No Target Control (NTC)	Experimental
Component	Amount (µL)	Amount (µL)	Amount (µL)
Nuclease-free H_2O	8	8	8
LavaLAMP™ DNA Master Mix (2X)	12.5	12.5	12.5
Target-Specific Primer Mix, 10X	--	2.5	2.5
DNA Positive Control LAMP Primer Mix	2.5	--	--
Green Fluorescent Dye *(optional)*	1	1	1
Total volume	**24**	**24**	**24**

4. Mix the added reagents completely by pipetting.
5. Dispense 24 µL each reaction mix into PCR tubes or a 96-well PCR plate.
   ***Note**: In order to minimize cross-contamination, perform steps 6-8 in an area separate from area where you are preparing reaction mix.*
6. Add 1 µL of Target DNA or Positive Control DNA to the appropriate reaction tubes or wells and 1 µL of nuclease-free water to the NTC reaction tubes or wells. Mix completely by pipetting.
7. Cap tubes or seal plate wells. Centrifuge briefly to collect contents prior to incubation.
8. Incubate the reactions as follows:

Step	Temperature	Time
Optional: Preheat	90˚C	≤5 min
1. Amplification	**Experimental and NTC**: 68˚C – 74˚C **Positive Control**: 74˚C	30 - 60 minutes
2. Hold (Optional)	4˚C	∞

9. Immediately stop amplification reactions using one of the three methods below. This step is required to stop the enzyme activity.
   a. Hold on ice or at 4°C.
   b. Add gel loading dye that produces a final 10 mM EDTA concentration.
   c. Perform a heat-kill step in a thermocycler or heat block at 95°C for 5 minutes
10. Detect amplified product using your detection method of choice.

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