QUICK PROTOCOL

LavaLAMP™ DNA Component Kits

(CAT. NO. 30076-1, 30077-1)

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. Centrifuge briefly to collect contents.
3. Prepare reaction mix(es) in the order listed below (Table 1).

   Notes:
   - *The reaction conditions recommended (Table 1) are for the use of Green Fluorescent Dye included with Cat. No. 30077-1 and 1 µL of Target DNA Sample. Adjust the volume of nuclease-free H_2O when using other dye or target DNA sample amounts.*
   - *Table 1 provides volumes for a single reaction, if multiple reactions are required increase volumes proportionately. Prepare enough reaction mix cocktail(s) for the number of amplification reactions being performed plus an additional 10% to accommodate slight pipetting errors.*

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

Component	Positive Control	No Target Control (NTC)	Experimental
	Amount (µL)	Amount (µL)	Amount (µL)
Nuclease-free H_2O	13.7	13.7	13.7
10X LavaLAMP™ DNA Buffer	2.5	2.5	2.5
LavaLAMP™ DNA Enzyme	1.0	1.0	1.0
dNTP mix, 25 mM	0.8	0.8	0.8
Magnesium Sulfate, 100 mM	2.5	2.5	2.5
Green Fluorescent Dye (optional)	1.0	1.0	1.0
Target-Specific Primer Mix, 10X	--	2.5	2.5
DNA Positive Control LAMP Primer Mix	2.5	--	--
Total	24.0	24.0	24.0

4. Mix the reagents completely by pipetting.
5. If more than one reaction is being run, dispense 24 µL of the reaction mix for each reaction into PCR tubes or a 96-well PCR plate.

   ***Note**: To minimize cross-contamination, perform steps 6-8 in an area separate from the area used to assemble the reaction mix.*
6. Add 1 µL of Target DNA or Positive Control DNA to the appropriate reaction tubes or wells. Add 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.
8. Incubate the reactions as follows:

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

9. Immediately stop the amplification reactions by inactivation, using one of the three methods below.
   a. Place on ice or at 4°C.
   b. Add gel loading dye to yield 10 mM EDTA (final concentration.)
   c. Heat-kill in a thermocycler or heat block at 95°C for 5 minutes
10. Detect amplified product using your method of choice.

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