QUICK PROTOCOL

Lucigen®
Simplifying Genomics

LavaLAMP™ RNA Component Kit

(CAT. NO. 30096-1, 30097-1)

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) use the Green Fluorescent Dye included with Cat. No. 30097-1 and 1 µL of Target RNA Sample. Adjust the volume of nuclease-free H_2O when using other dye or target RNA 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	14.95	14.95	14.95
10X LavaLAMP™ RNA Buffer	2.5	2.5	2.5
LavaLAMP™ RNA Enzyme	1.0	1.0	1.0
dNTP mix, 25 mM	0.8	0.8	0.8
Magnesium Sulfate, 100 mM	1.25	1.25	1.25
Green Fluorescent Dye (optional)	1.0	1.0	1.0
Target-Specific Primer Mix, 10X	--	2.5	2.5
RNA 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 RNA or Positive Control RNA 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. Amplify and detect product using your method of choice below. Incubate reactions as follows:

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

   a. For real-time monitoring of fluorescence using the included Green Fluorescent Dye, us the FAM or SYBR Green wavelength settings on a real-time instrument and take readings every 15-30 seconds for 30-60 minutes.

   b. For end-point detection, immediately stop amplification reactions using one of the three methods below. This step is required to stop enzyme activity.
      i. Hold on ice or at 4°C.
      ii. Add gel loading dye to yield a final concentration of 10 mM EDTA.
      iii. Perform a heat-kill step in a thermocycler or heat block at 95°C for 5 minutes.

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