QUICK PROTOCOL

Lucigen
Simplifying Genomics

LavaLAMP™ RNA Master Mix

(CAT. NO. 30086-1, 30087-1)

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). When possible, make enough of the reaction mixes for all reactions at one time.

   ***Notes**:*
   - *The reaction conditions recommended (Table 1) are for the use of Green Fluorescent Dye included with Cat. No. 30087-1 and 1 µL of Target DNA Sample. Adjust the volume of nuclease-free H_2O when using other amounts of dye or target RNA sample.*
   - *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

Component	**Positive Control**	**No Target Control (NTC)**	**Experimental**
	Amount (µL)	Amount (µL)	Amount (µL)
Nuclease-free H_2O	8	8	8
LavaLAMP™ RNA Master Mix (2X)	12.5	12.5	12.5
Target-Specific Primer Mix, 10X	--	2.5	2.5
RNA 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. If more than one reaction is being run, dispense 24 µL of each reaction mix 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 set up 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 to incubation.
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, use 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.

Lucigen Corporation
Technical Support: (888) 575-9695 | (608) 831-9011 | techsupport@lucigen.com

MA175 Feb2018