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LavaLAMP RNA Master Mix

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LavaLAMP RNA Master Mix

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1. Introduction

The [LavaLAMP™ RNA Master Mix](#) is intended to simplify development and optimisation of RNA LAMP (loop-mediated isothermal amplification) reactions. LAMP kits are commonly available as multi-component kits that require optimisation (e.g., MgSO₄, betaine, enzyme, reaction temperature, primer concentration). The LavaLAMP RNA Master Mix greatly simplifies reaction optimisation by limiting optimisation to target-specific components and conditions (LAMP primers, target concentration and reaction temperature).

2. Product designations and kit components

Product	Kit size	Catalogue number	Reagent description	Part number	Volume
LavaLAMP RNA Master Mix	200 reactions	30086-1	LavaLAMP RNA Master Mix	F824234-1	2 x 1.25 mL
			RNA Positive Control LAMP Primer Mix	F814233-1	25 µL
			RNA Positive Control	F824232-1	10 µL
LavaLAMP RNA Master Mix with Dye	200 reactions	30087-1	LavaLAMP RNA Master Mix	F824234-1	2 x 1.25 mL
			RNA Positive Control LAMP Primer Mix	F814233-1	25 µL
			RNA Positive Control	F824232-1	10 µL
			Green Fluorescent Dye	F883827-1	200 µL
Green Fluorescence Dye	200 reactions	30078-2	Green Fluorescence Dye	F883827-1	200 µL

Table 1. LavaLAMP RNA Master Mix kits and constituent components.

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3. Product specifications

Storage: Store all kits and components at -20 °C.

Quality control:

- **Absence of endonuclease**

LavaLAMP RNA Master Mix is determined to be free of detectable endonuclease or nicking activity. One µg of supercoiled plasmid DNA is incubated with LavaLAMP RNA Master Mix for 16 hours at 70 °C. Reactions are analysed by agarose gel electrophoresis. With the LavaLAMP RNA Master Mix deemed to be free of endonuclease or nicking activity if there is no alteration in mobility.

- **Absence of exonuclease**

LavaLAMP RNA Master Mix is tested to be free of contaminating exonuclease activity by incubating 1 µg of Hind III-digested lambda DNA with LavaLAMP RNA Master Mix at 70 °C for 16 hours. Reactions are analysed by agarose gel electrophoresis, and the enzyme is deemed to be free of exonuclease activity if there is no alteration in mobility.

- **Functional assays**

LavaLAMP RNA Master Mix Amplification system is tested for performance by isothermal amplification of a target region within the MS2 bacteriophage genome.

4. General considerations

4.1 Materials supplied by the user

- 10x target-specific LAMP primer mix
 - Typical 10x formulation:
 - 2 µM each, forward outer primer (F3) and backward outer primer (B3)
 - 8 µM each, forward loop primer (FL) backward loop primer (BL)
 - 16 µM each, forward inner primer (FIP) and backward inner primer (BIP)
- Target RNA: Most routine methods of RNA target purification are sufficient (e.g., phenol/chloroform or guanidine/silica-based methods), and LAMP reactions generally tolerate some contaminants in the RNA sample. However, trace amounts of purification reagents (e.g., phenol, Proteinase K, ethanol, etc.) may inhibit amplification; therefore, addition of large amounts of sample should be avoided. In addition, EDTA can inhibit amplification, so it is preferable to dissolve the RNA target in water or EDTA-free buffer. If TE must be used, we recommend using low TE (10 mM Tris, 0.1 mM EDTA).
- Thermal cycler or heat block: A thermal cycler with a heated lid is ideal to prevent evaporation of the reaction mix. If no such lid is available, the reaction mixture can be overlaid with one-half reaction volume of PCR-grade mineral oil, but mineral oil may slow the reaction. We recommend using calibrated instruments.
- Real-time instrument or fluorometer (if using Green Fluorescent Dye for detection – see section 6).

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4.2 Positive controls

The positive controls provided with all our LavaLAMP kits are designed to be used for two main purposes. These are i) initial verification of laboratory set up and processes for new users of LAMP, and ii) troubleshooting if you are experiencing any issues with your reactions.

If you are completely new to LAMP, we recommend using the positive control reactions that are provided to set up a trial reaction before you begin working with your own RNA and assays. Each kit includes the necessary RNA template and LavaLAMP assay primers.

A LavaLAMP reaction involving the provided positive control should be set up as detailed in table 2.

Component	Volume (µL)
Nuclease-free water	8
LavaLAMP RNA Master Mix	12.5
RNA positive control LAMP primer mix	2.5
Green Fluorescent Dye (optional) (If not using, replace volume with nuclease-free water)	1
Positive control RNA	1
Total Reaction volume	25

Table 2. Reaction set up for LavaLAMP RNA positive control reaction.

The RNA positive control requires amplification to be performed at 68 °C for 30 to 60 minutes.

The positive control can also be used for troubleshooting issues with your own LavaLAMP assays. Running a positive control reaction will enable you verify that all components other than the designed primers are functioning correctly.

Please note that it may not be possible to run a positive control reaction alongside your own LavaLAMP assays as designed because the control must be amplified at a specific temperature. We do not advise amplifying the positive control reactions at any other temperature.

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4.3 Primer design

LAMP commonly employs a set of six primers which must be supplied by the user. LGC Biosearch Technologies recommends using previously established designs or designing new primer sets. Below are two tools that you may find helpful for primer design:

- LAMP Designer by Premier Biosoft is available for purchase at:
<http://www.premierbiosoft.com/isothermal/lamp.html>
- PrimerExplorer by Eiken is a free online application that can be accessed at:
<https://primerexplorer.jp/e/>

Detailed guidance on primer design and on performing primer design screens can be found in section 5 and section 6 of our [LavaLAMP: technical guide for new users](#) respectively.

5. Reaction setup

The LavaLAMP RNA Master Mix contains all components required for amplification, including magnesium sulfate (MgSO_4).

An NTC reaction should be run alongside experimental samples.

Biosearch Technologies recommend preparing sufficient reaction mix for the number of individual reactions that are to be performed, with an additional 10% to accommodate for pipetting inaccuracies.

LAMP reactions are very sensitive to target RNA or amplicon carryover contaminants which can result in false positive amplification results. To prevent contamination of your LAMP reactions with target RNA or target amplicons, designate and use an area for reaction setup that has never been exposed to the target RNA or amplified products. Then use a different area to add your target RNA to your reactions (steps 6-8) that has never been exposed to amplified material. Finally, analyse LAMP reaction products (section 6) in an area separate from both reaction setup areas.

5.1 Before you start

1. Always wear gloves while handling components. Set up reactions using good laboratory techniques that minimise cross contamination.
2. Thaw and hold reagents on ice and set up reactions on ice to avoid background amplification.
3. Calculate the total volume of each reagent required for the planned experiment and verify that sufficient volume is available before proceeding to reaction setup.

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4. Set a thermal cycler or heat block to the desired temperature. If using a heat block, we recommended using 0.2 mL PCR tubes and monitoring the temperature closely.
5. Biosearch Technologies encourages all users to perform a No Template Control (NTC) reaction with each primer set.

5.2 Step-by-step protocol

1. Thaw all kit components on ice. All LavaLAMP RNA Master Mix reactions should be set up on ice and maintained at 4 °C prior to amplification.
2. Mix each component thoroughly before use by vortexing each tube for three to ten seconds and then centrifuge briefly at 4 °C to collect contents.
3. Prepare initial reaction mixes in a single tube in the order listed in table 3. During this step, the reaction mixes and all reaction tubes/plates should be kept on ice to reduce non-specific amplification. Primers should be added just prior to target addition and incubation.

Component	NTC	Experimental
	Volume (µL)	Volume (µL)
Nuclease-free water	8	8
LavaLAMP RNA Master Mix (2X)	12.5	12.5
Target Specific Primer Mix, 10X	2.5	2.5
Green Fluorescent Dye (optional)	1	1
Total volume	24	24

Table 3. Reaction setup for LavaLAMP RNA Master Mix reactions. Reactions assume Green Fluorescent Dye (supplied with Cat. No. 30067-1) and 1 µL of target RNA sample are being used. If you are not using Green Fluorescent Dye and/or a different volume of target RNA is being used, adjust the volume of nuclease-free water accordingly.

4. After all reagents have been added, mix the combined reaction components by pipetting. This step is required to ensure uniform distribution of all reaction components in the reaction mix.
5. Dispense 24 µL of the prepared reaction mix(es) into the required number of PCR tubes or a 96-well PCR plate.
6. Add 1 µL of target RNA (or positive control template RNA if running positive control reactions) to the appropriate reaction tubes or wells and 1 µL of nuclease-free water to the NTC reaction tube(s) or well(s). Mix completely by pipetting. Cap tubes or seal plate wells. Centrifuge briefly to collect contents prior to incubation.
8. Using a heat block or thermal cycler, incubate the reactions as detailed in table 4. Reactions should be transferred directly from ice to a pre-heated heat block or thermal cycler set and equilibrated to the correct reaction temperature.

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Step	Temperature	Time
1. Amplification	Experimental and NTC: 68-74 °C RNA positive control template: 68 °C	30-60 minutes
2. Hold (optional)	4 °C	∞

Table 4. Temperature requirements for the LavalAMP RNA reaction. The amplification threshold is usually reached in 8-20 minutes. As a result, 30 minutes is the recommended incubation time for end-point reactions. Longer incubation times may lead to the appearance of undesired background (see figure 3 for an example).

9. Immediately stop amplification reactions using one of the three methods below. This step stops 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 thermal cycler or heat block at 95 °C for 5 minutes.

Note: Amplified reactions may be kept at -20 °C for long term storage.
10. Detect amplified product – see section 6 for details.

6. LAMP assay detection

For LAMP assay detection, there are three main methods we would recommend:

- For fluorescent assays in real-time detection instruments: Monitor fluorescence using the FAM or SYBR Green channels at 15-30 second intervals for 30-60 minutes.
- For fluorescent or non-fluorescent end-point assays: Immediately stop enzyme activity by one of the methods in the previous section and then assay using your detection method of choice:
 - a. Fluorescent assays in real-time detection instruments: monitor reaction fluorescence using the FAM channel to detect amplified product.
 - b. End-point fluorescent assays: measure fluorescence in a fluorometer using the FAM channel to detect amplified product.
 - c. Non-fluorescent end-point assays: agarose gel (visual) or spectrophotometer (turbidity, OD600). Note: turbidity is a low-cost option and is field deployable but is single-use only and not as sensitive as other methods. Agarose gel is more applicable for troubleshooting e.g. determining whether NTC amplification is due to contamination or primer dimerisation.

6.1 Green Fluorescent Dye

While not required, we recommend using Green Fluorescent Dye to detect amplified RNA. Green Fluorescent Dye is packaged separately from the master mix. Do not combine the dye with the master mix until you are setting up the reaction.

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Detection of Green Fluorescent Dye requires a real-time amplification instrument or a fluorometer for end-point analysis. This should be capable of excitation at 490 nm and measuring fluorescence at 520 nm.

- The following instruments have been successfully used with Green Fluorescent Dye: AmpliFire (Agdia), CFX96 and iQ5 Thermal Cyclers (Bio-Rad), ESEQuant TS2 (Qiagen), Genie II (OptiGene), and the ABI 7500 Real-Time PCR System (Thermo Fisher Scientific).
- Fluorescent dyes such as V13-01184, EvaGreen and SYTO-13 have also been used successfully with the LavaLAMP RNA Component Kit, but SYBR Green Dye will not work acceptably. Optimisation of dye concentration is necessary to produce the fastest TTR (time to results).

7. Typical LAMP results

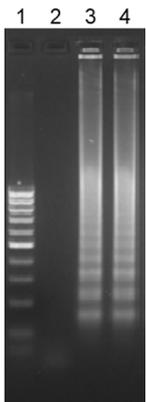


Figure 1: Reaction products from a Positive Control Target.
Lane 1: 100 bp marker,
Lane 2: No Template Control reaction.
Lanes 3 and 4: A distinct banding pattern is seen among the smear, which is indicative of a positive LAMP reaction.

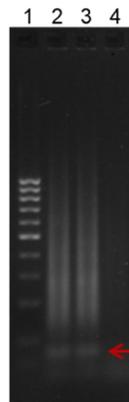


Figure 2: Typical background amplification in a LAMP reaction.
Lane 1: 100 bp ladder,
Lanes 2 and 3: Non-specific or background amplification appears as a smear of DNA fragments with no visible or distinct bands. A prominent primer dimer band is also characteristic of non-specific amplification.
Lane 4: Absence of non-specific amplification (no products).

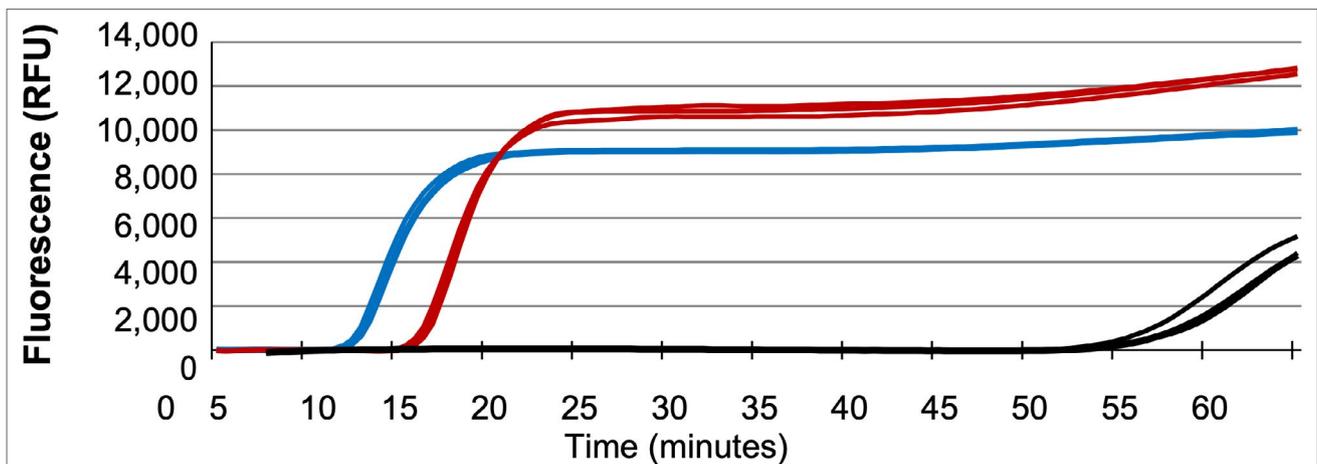


Figure 3: Early fluorescent signals from positive LAMP reactions and late background signals from NTC Controls after extended incubation. LAMP reactions were run in a real-time thermal cycler and the fluorescent signal from each reaction was captured over a 60-minute reaction time. The red and blue lines represent the fluorescent signals from Positive Control reactions with varying amounts of target. The black lines are the non-specific background amplification signals that can arise later in a No Target Control (primers) reaction.

8. Further support

If you require additional support, please contact our technical support team at techsupport@lgcgroup.com.



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