Lucigen®
Simplifying Genomics

LavaLAMP™ DNA Master Mix

Please read carefully and thoroughly before beginning

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Technical Support

Lucigen is dedicated to the success and satisfaction of our customers. Our products are tested to assure they perform as specified when used according to our recommendations. It is imperative that the user supplied reagents are of high quality. Please follow the instructions carefully and contact our technical service representatives if additional information is necessary. We encourage you to contact us with your comments regarding the performance of our products in your applications. Thank you.

Lucigen Technical Support:
Email: techserv@lucigen.com
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Product Guarantee: Lucigen guarantees that this product will perform as specified for one year from the date of shipment. Please avoid using reagents for greater than one year from date of receipt.

Product Description

The LavaLAMP™ DNA Master Mix is intended to simplify development and optimization of DNA LAMP (loop-mediated isothermal amplification) reactions. LAMP kits are commonly available as multi-component kits that require optimization (e.g. $MgSO_4$, betaine, enzyme as well as temperature, primer concentration, etc.). The LavaLAMP™ DNA Master Mix greatly simplifies reaction optimization by limiting optimization to target specific components/conditions (LAMP primers, target concentration and reaction temperature). Additionally, this master mix is heat stable at 90˚C for ≤5 minutes, which enables the addition of a reaction preheating step to help reduce the time to result (TTR) when using purified target

DNA as input. This kit is for research purposes only, and available under the limited-use license described at the end of this document.

LAMP commonly employs a set of six primers (F3, B3, Loop-F, Loop-B, FIP and BIP), which must be supplied by the user. Lucigen recommends using previously-established designs or designing new primer sets using the Eiken web utility (see Appendix A). Not all primer sets identified by this program are guaranteed to perform with LavaLAMP™ DNA Master Mix or any other LAMP system. We recommended that you design multiple primer sets to identify the best performing set. We also highly recommend inclusion of loop primers (Nagamine, 2002).

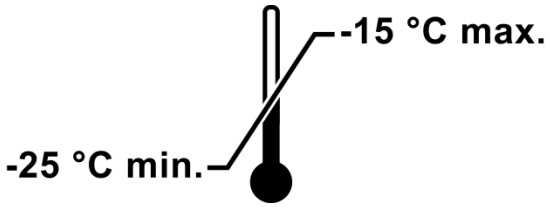
LavaLAMP™ DNA Master Mix amplified products may be detected by agarose gel electrophoresis or by real-time/end-point monitoring using fluorescent double-stranded DNA-binding dyes, such as the Green Fluorescent Dye included in Cat. No. 30067-1. Turbidity may also be used (Mori, 2001), but this method is less sensitive.

Product Designations and Kit Components

Product	Kit Size	Catalog Number	Reagent Description	Part Numbers	Volume
LavaLAMP™ DNA Master Mix	200 Reactions	30066-1	LavaLAMP™ DNA Master Mix	F833734-1	2 x 1.25 mL
			DNA Positive Control LAMP Primer Mix	F813735-1	25 µL
			DNA Positive Control	F823736-1	10 µL
LavaLAMP™ DNA Master Mix with Dye	200 Reactions	30067-1	LavaLAMP™ DNA Master Mix	F833734-1	2 x 1.25 mL
			DNA Positive Control LAMP Primer Mix	F813735-1	25 µL
			DNA Positive Control	F823736-1	10 µL
			Green Fluorescent Dye	F883827-1	200 µL

Components and Storage

Store all kits and components at -20 °C



Materials Supplied by the User

- 10X target-specific LAMP primer mix
  - Common/recommended 10X formulation:
    2 µM each, F3 and B3 primers
    8 µM each, Loop-F and Loop-B primers
    16 µM each, FIP and BIP primers,
  - **Note**: See LAMP Reaction Optimization section for additional details on primers
- Target DNA
- Thermocycler or heat block (recommend using calibrated instruments)

- **Optional**: If using the Green Fluorescent Dye included in Cat. No. 30067-1 for detection of amplified DNA, a real-time instrument or fluorometer for end-point analysis capable of measuring the detection dye will be required. Other fluorescent dyes may be used in these assays, but optimization of dye concentration will be necessary to produce the fastest times to results. EvaGreen and SYTO-13 have been used successfully with this master mix.

  Note: Lucigen has used the following instruments to successfully detect amplified product by fluorescence in combination with the Green Fluorescent Dye: AmpliFire (Douglas Scientific), CFX96 and iQ5 Thermocyclers (Bio-Rad), ESEQuant TS2 (Qiagen), Genie II (OptiGne), ABI 7500 Real-Time PCR System (Thermo Fisher Scientific).

Before You Start:

1. Always wear gloves while handling components. Set up reactions using good laboratory techniques that minimize 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 enough reagent is available before proceeding to reaction setup.
4. Set a thermocycler 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. Lucigen strongly encourages all users to perform a No Target Control (NTC) reaction and new users to also include a Positive Control reaction to aid in troubleshooting.

Reaction Setup

The LavaLAMP™ DNA Master Mix contains all components required for amplification, including Magnesium Sulfate ($MgSO_4$). If you include the Green Fluorescent Dye in the reactions, you can detect the amplified products using a real-time instrument or fluorometer. Alternatively, you can detect amplified products by agarose gel electrophoresis or turbidity using a spectrophotometer. For most targets, optimization of reaction temperature and primer and/or target concentrations will result in faster time to result and reduced background amplification (see LAMP Reaction Optimization section for optimization recommendations).

***Note**: In order to minimize cross-contamination, perform steps 6-8 in an area separate from the area where you are preparing reaction mix.*

1. Thaw all kit components on ice and keep them on ice as you set up the reactions.
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 below in Table 1. During this step the reaction mixes and all reaction tubes/plates should always be held on ice to reduce non-specific amplification/background. The No Target Control (NTC) reaction using the Target-specific primer set(s) is strongly recommended to demonstrate a lack of background amplification within the reaction time(s) tested.

   ***Notes:** 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.*

   When possible, we recommend preparing 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

	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. After all reagents have been added, mix the reaction completely by pipetting. This step is required to ensure uniform distribution of all reaction components.
5. Dispense 24 µL of the Experimental and Control reaction mixes for each reaction 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 where you prepared the 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. Using a heat block or thermocycler, incubate the reactions as follows:

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

[a] This master mix is heat stable at 90˚C for ≤5 minutes. This preheating step may reduce time to result (TTR) when using purified DNA as target input. This preheating step is not recommended when using crude starting samples (e.g. cells, blood, etc.).

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 thermocycler 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 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), spectrophotometer (turbidity, OD_{600})

LAMP Reaction Optimization

Temperature Optimization

68˚C is the recommended initial reaction temperature when testing new primer sets with the LavaLAMP™ DNA Master Mix.

For additional temperature optimization:

1. Set up a series of identical LAMP reactions in tubes of plates using the Experimental reaction conditions and run the reactions from 68°C – 74°C. Lucigen does not recommend reaction temperatures above 74°C or below 68°C.
2. Run the reactions in a calibrated thermocycler or heat block.
3. Be sure to determine the optimal temperature for each LAMP Primer Set being tested.

Note that LavaLAMP™ DNA Master Mix cannot be used with PCR or Bst DNA Polymerase reaction conditions.

Target-specific LAMP Primer Mix Design, Quality, and Concentration

As mentioned previously, Lucigen strongly recommends designing and testing multiple LAMP primer sets because primer design is one of the most important factors in optimizing LAMP results. For details on primer design, please see Appendix A.

We recommend HPLC purification for the FIP and BIP primers and standard desalting for the Loop, F3 and B3 primers. For optimal results, HPLC purify all primers. Primers can be resuspended in nuclease-free water or low TE (10 mM Tris-Cl, 0.1 mM EDTA, pH 8.0).

The recommended starting Target-specific LAMP Primer Mix, 10X is:

2 µM each, F3 and B3 primers
8 µM each, Loop-F and Loop-B primers
16 µM each, FIP and BIP primers

Depending on the primer-template system, it may be necessary to optimize primer concentration after the optimal reaction temperature is identified. Certain primer sets may be prone to background amplification at or near the commonly used LAMP primer concentrations. If undesired background amplification is observed, a primer concentration titration from 0.2X – 1X should be performed. The concentration of all primers may be adjusted in unison, by adding varying amounts of the Target-specific LAMP Primer Mix, 10X. Reducing the primer concentration may reduce sensitivity and reaction yield, or it may increase the time required to amplify your target. Lucigen does not recommend increasing primer concentration above the recommended levels because it generally leads to increased background amplification.

Target DNA and Dilution Buffer

We recommend starting your LAMP assay development using purified target DNA as input. Once optimization is complete, then you can start testing different sample types knowing that your assay (primers, temperature) works with clean, purified DNA target.

Preparation of target dilutions in 25 mM Tris-Cl (pH 8.60) may help increase sensitivity. When using Tris-Cl as dilution buffer, it is very important to adjust pH to 8.60 ($\pm$ 0.05) and filter the buffer to remove any contaminants. Prepared buffer solution can be stored at room temperature, however for long-term storage and to avoid contamination, aliquot the buffer and store at 4°C.

Typical LAMP Results

Correct Target-specific Amplification

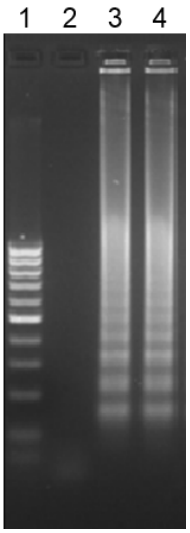


Figure 1: Positive LAMP Reaction Products from a Positive Control Target. Lane 1: 100 bp Marker, Lane 2: No Target Control reaction. Lanes 3 and 4: A distinct banding pattern is seen among the smear, which is indicative of a positive LAMP reaction.

Spurious Background Amplification

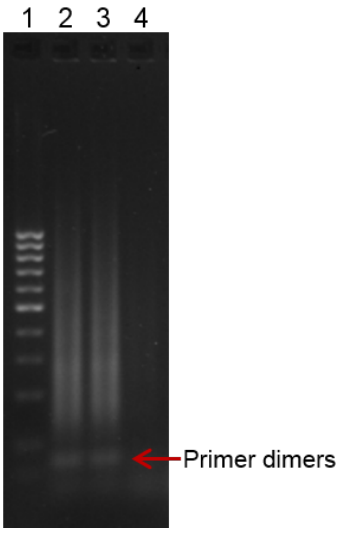


Figure 2: Typical Background Amplification in a LAMP Reaction. Lane 1: 100 bp ladder, Lanes 2 and 3: Non-specific or background amplification appears as a smear of DNA fragments with no visible or distinct bands. A prominent primer dimer band is also characteristic of non-specific amplification. Lane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

Fluorescent Signals from Different LAMP Reactions

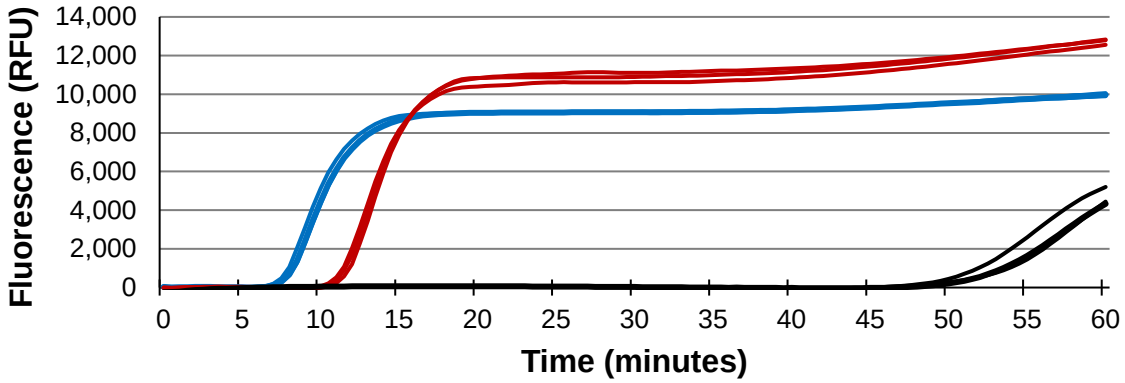


Figure 3: Early Fluorescent Signals from Positive LAMP Reactions and Late Background Signals from NTC Controls after Extended Incubation. LAMP reactions were run in a real-time thermal cycler and the fluorescent signal from each reaction was captured over a 60 minute reaction time. The red and blue lines 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.

Additional Amplification Guidelines

Prevent Target DNA Contamination

LAMP reactions, much like PCR reactions, are very sensitive to target DNA or amplicon carryover contaminants which can result in false positive amplification results. In order to prevent contamination of your LAMP reactions with target DNA or target amplicons, designate and use an area for reaction setup that has never been exposed to the target DNA or amplified products. Then use a different area to add your target DNA to your reactions that has never been exposed to amplified material. Finally, analyze LAMP reaction products in an area separate from both of these reaction setup areas.

Cold Reaction Setup

The LavaLAMP™ DNA Master Mix exhibits residual activity above 4°C that can cause non-specific background amplification at reaction temperatures below specific reaction temperature of 68-74°C.

- All LavaLAMP™ DNA Master Mix reactions should be set up on ice and maintained at 4°C prior to amplification.
- Primers should be added just prior to target addition and incubation.
- To start amplification, the reactions should be transferred directly from ice to a pre-heated heat block or thermal cycler set and equilibrated to the correct reaction temperature.

Target Preparation

Most routine methods of target purification are sufficient (e.g. phenol/chloroform or guanidine/silica-based methods), and LAMP reactions are generally more tolerant to some contaminants in the DNA sample. However, trace amounts of purification reagents (e.g. phenol, Proteinase K, ethanol, etc.) may inhibit amplification. In addition, EDTA can inhibit amplification, and as such, it is preferable to use nucleic acid target that is dissolved in water or EDTA-free buffer rather than standard TE (10 mM Tris, 1 mM EDTA). If TE must be used, we recommend using low TE with 0.1 mM EDTA.

LAMP Reaction Timing

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 Fig. 3 for an example).

Reaction Overlay

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.

Appendix

A: Primer Design Software

The Eiken PrimerExplorer software is a free online software application that will assist users in designing a LAMP primer set. The software can be accessed at the URL listed below. As a reminder, we recommend testing multiple LAMP Primer sets in order to identify the best performing set.
PrimerExplorer link: https://primerexplorer.jp/e/.

Another software option is LAMP Designer by Premier Biosoft, which is available for purchase at http://www.premierbiosoft.com/isothermal/lamp.html.

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B: Quality Control Assays

Absence of Endonuclease

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

Absence of Exonuclease

LavaLAMP™ DNA Master Mix is tested to be free of contaminating exonuclease activity by incubating 1 µg of Hind III-digested lambda DNA with master mix at 70°C for 16 hours. Reactions are analyzed 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™ DNA Master Mix Amplification system is tested for performance by isothermal amplification of a target region within the M13mp18 ssDNA genome.

C: References and Additional Reading

Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, Hase T. Loop-mediated isothermal amplification of DNA. Nucleic Acids Res. 2000 28(12):E63.

Nagamine K, Hase T, Notomi T. Accelerated reaction by loop-mediated isothermal amplification using loop primers. Mol Cell Probes. 2002 16(3):223-9.

Mori Y, Nagamine K, Tomita N, Notomi T. Detection of loop-mediated isothermal amplification reaction by turbidity derived from magnesium pyrophosphate formation. Biochem Biophys Res Commun. 2001 289(1):150-4.

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