Lucigen®
Simplifying Genomics

OmniAmp® RNA & DNA LAMP Kit

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 reagents supplied by the user, especially the RNA specimens to be amplified, are of the highest 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:
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Phone: (888) 575-9695

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 receipt.

Product Description

The OmniAmp™ RNA and DNA LAMP Kit is intended to simplify development of LAMP (loop-mediated isothermal amplification) reactions for detecting RNA or DNA. LAMP is a commonly used isothermal amplification system that was developed and patented by Eiken Chemical Co. (see Appendix C). This kit may be used for research purposes only, under the limited-use license described at the end of this document. Details of the LAMP reaction and its use can be found in the References section or Appendix A.

OmniAmp DNA polymerase is unique in having both reverse transcription and strand-displacing activities. This rare and powerful combination enables LAMP detection of either DNA or RNA targets. Other isothermal amplification techniques that rely on strand displacement may also be possible with OmniAmp reagents, however they are not tested.

LAMP commonly employs a set of six primers, 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 OmniAmp or any other enzyme system. It is recommended that two or three primer sets be designed and compared experimentally. We highly recommend inclusion of loop primers (Nagamine, 2002).

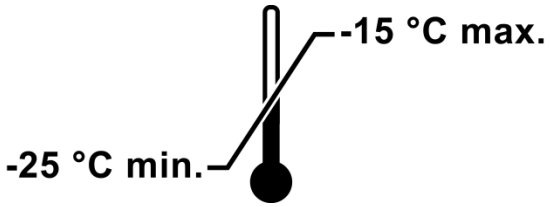
OmniAmp LAMP products may be detected by agarose gel electrophoresis, or by real-time monitoring using double-stranded DNA-binding fluorescent dyes, such as EvaGreen® (Biotium). Turbidity may also be used (Mori, 2001), but this method is less sensitive.

Product Designations

Product	Kit Size	Catalog number	Reagent Description	Part Numbers	Volume
OmniAmp™ RNA & DNA LAMP Kit	100 Reactions	30065-1	OmniAmp DNA Polymerase, 50X	F831942-1	50 µL
			10X DNA Polymerase Buffer C	F881958-1	1 mL
			Magnesium Sulfate 100 mM	F98695-1	1 mL
			Betaine, 5 M	F881901-1	1 mL
			RNA Control I LAMP Primer Mix, 10X	F812344-1	20 µL
			Nuclease-free Water	F98698-1	1 mL
			Positive Control	F823398--1	20 µL
	500 Reactions	30065-2	OmniAmp DNA Polymerase, 50X	F831942-1	5 x 50 µL
			10X DNA Polymerase Buffer C	F881958-1	5 x 1 mL
			Magnesium Sulfate 100 mM	F98695-1	5 x 1 mL
			Betaine, 5 M	F881901-1	5 x 1 mL
			RNA Control I LAMP Primer Mix, 10X	F812344-1	5 x 20 µL
			Nuclease-free Water	F98698-1	5 x 1 mL
			Positive Control	F823398-1	5 x 20 µL

Components and Storage

Store all kits and components at -20 °C



Material to be Supplied by the User

- dNTP mix, 25 mM each
- 20X EvaGreen® Dye (Biotium)
- Target-specific 10X LAMP Primer mix
  (Common formulation: 16 µM FIP and BIP primers, 8 µM Loop-F and Loop-B primers and 2 µM F3 and B3 primers)
- Target RNA or DNA

Before you start:

1. Always wear gloves while handling the components.
2. Verify sufficient volume of kit components required for planned reactions prior to setup.
3. Make sure that thermocycler or heat block is set to the desired temperature. Note that when using a heat block, it is recommended to use 0.2 mL PCR tubes and to monitor the temperature closely.
4. RNase free environment and procedures should be used to avoid contamination.
5. Add target in an area separated from the area where the reaction mix is prepared.
6. Thaw reagents and set up reactions on ice.
7. Reaction setup should be done using good laboratory techniques that minimize cross contamination.

Note: First-time users are strongly encouraged to perform the control reaction as described below in order to better familiarize themselves with LAMP and the OmniAmp system.

Reaction Setup

Target-Specific Optimization

OmniAmp polymerase is provided with Polymerase Buffer C, which is designed to support LAMP and other isothermal amplification processes. Buffer C contains all components required for amplification, including Magnesium Sulfate ($MgSO_4$) at 2 mM final concentration. However, certain targets and amplification systems will require optimization using the included $MgSO_4$and Betaine supplements. For most targets, optimization of Magnesium and Betaine concentrations will result in shorter time to result and reduced background amplification.

Experimental Reaction:

Component	Final Concentration or Quantity	Volume, µL	Recommended Concentrations
Nuclease-free H_2O	n/a	To 25 µL	n/a
10X DNA Polymerase Buffer C[1]	1X	2.5	n/a
dNTPs (25 mM each)[2]	800 µM (recommended)	0.8 µL	n/a
100 mM $MgSO_4$	2 - 12 mM[4]	0 - 2.5 µL	8 mM (2 µL)
5 M Betaine[2]	0.2 - 1.0 M	1 - 5 µL	0.15 M (0.75 µL)
Target-Specific Primer Mix[3]	Variable	Variable	n/a
OmniAmp DNA Polymerase, 50X	1X - 2X	0.5 - 1.0 µL	1X
Template RNA or DNA	0.01ng - 100 ng	Variable	1X (0.5 µL)
Total volume		**25**	n/a

1. Buffer C is prepared with low magnesium (2 mM final) to allow optimization. However, most LAMP systems will require a final $MgSO_4$ concentration of 6 mM or greater.
2. It is strongly recommended to use dNTPs that have not undergone multiple freeze-thaw cycles. LAMP systems are more sensitive to dNTP quality than typical PCR systems, so fresh dNTP's are recommended for applications where sensitivity and reproducibility are important.
3. Please see appendix A for LAMP Primer design resources.

Note: If you plan to include additional reagents, such as those needed for quantitation of the reaction product, reduce the amount of nuclease-free water used accordingly. It is recommended to prepare a reaction mix 10% greater than required number of reactions to account for overage.

Note: If a dye is added for amplification quantitation, use between 0.2 µL and 0.5 µL per reaction, or other per the manufacturer's recommendations. Excessive amounts of dye will interfere with or inhibit the reaction.

Control Reaction

Table 1. Control Reaction Setup

Component	Final Concentration or Quantity	Volume, µL
Nuclease-free H_2O	--	14.45
10X DNA Polymerase Buffer C	1X	2.5
dNTPs (25 mM each)	800 µM	0.8
100 mM $MgSO_4$	8 mM	2.0
5 M Betaine	0.15 M	0.75
20X Dye	0.4X	0.5
LAMP RNA Control I Primer Mix, 10X	1X	2.5
OmniAmp DNA Polymerase, 50X	1X	0.5
Positive Control[1]	--	1
Total volume		**25**

Workflow:

In order to minimize cross-contamination, steps 6 and 7 should be done in an area separate from area where you are preparing reaction mix.

1. Thaw all kit components and hold on ice.
2. **All components should be mixed well before use. Vortex all tubes for 10 seconds then centrifuge briefly to collect.**
3. Prepare the reaction mix as shown in Table 1 in the order listed. Add all the components except the template (Positive Control). During this step the reaction mix tube should always be held on the ice to prevent background activity of enzyme.
4. After all reagents have been added, mix the reaction completely. This step is required to ensure uniform distribution of all reaction components.
5. Dispense 22.5 µL of reaction mix in a PCR tube or 96-well PCR plate for each reaction.
6. Add 1 µL of the Positive Control.
7. Cap tubes or seal plate wells. Centrifuge briefly to collect prior to incubation.
8. Incubate at 70 °C for 30 minutes.
9. Immediately stop amplification reactions using one of the three methods below. This step required to stop the enzyme activity.
    a. Hold on ice or at 4 °C.
    b. Add gel loading dye with 50 mM EDTA.
    c. Perform a heat-kill step in a thermocycler or heat block at 95 °C for 2 minutes.
10. Run samples on a 2% agarose gel.

Note: Reactions may be kept at -20 °C for longer term storage.

Isothermal Amplification Conditions

The following general program is recommended:

Step	Temperature	Time
1. Amplification	68 ˚C – 72 ˚C	20 - 35 Minutes[1]
2. Hold	4 ˚C	∞

1. The amplification threshold is usually reached in 8-20 minutes, depending on template concentration. However, long reactions can lead to undesired background. Please see the optimization notes below on reaction time.

LAMP Reaction Optimization

Lucigen recommends optimizing reaction conditions in two separate steps. In the first step, determine the optimal Magnesium and Betaine concentrations. Then use that buffer formulation over a range of temperatures to find the condition with the best overall performance.

Step 1: Magnesium and Betaine Concentration

Magnesium and Betaine concentrations can be easily optimized using an array of reactions run in parallel. For best results, use a 96-well plate in a calibrated thermocycler. For buffer optimization work, perform all reactions at 68 °C.

Note: Polymerase Buffer C already contains $MgSO_4$ at 2 mM, which is not sufficient for most reactions. Therefore most reactions will require supplemental magnesium sulfate. Use of buffers other than Buffer C provided in this kit is not recommended

Note: To allow more precise adjustments for individual reactions, the stock 5 M Betaine solution can be diluted to 1 M using nuclease-free water.

Using these suggested increments in a matrix of conditions, it should be possible to quickly find an approximately optimal reaction formulation. More precise optimization can be done in subsequent steps if needed .

Step 2: Temperature Optimization

After the optimum buffer composition has been established, determine the best temperature for your targets by performing the reaction at a range of temperatures. For most targets the optimal reaction temperature is between 66 °C and 72 °C. Reaction temperatures above 72 °C or below 64 °C are not recommended.

Note: Higher reaction temperatures generally provide faster amplification, but may also result in increased background (non-specific) amplification.

Other optimization notes

Reaction time and temperature

LAMP and other isothermal amplification processes are prone to spurious amplification if the reactions are allowed to proceed for too long or if they are run at too high a temperature. Therefore, during optimization it may be necessary to reduce time and temperature from apparently optimal conditions in order to avoid unwanted background amplification or decreased specificity. Reaction times of 30 minutes or less are strongly recommended. This is true of reactions with the target present as well as of no-template controls.

Enzyme Concentration

As with time and temperature, the use of more enzyme may result in better amplification results. Using the enzyme at up to 2X concentration may increase the reaction speed or sensitivity, however it can also lead to increased background amplification.

Primer Concentration

Depending on the primer-template system, it may be necessary to optimize primer concentration after the optimum reaction condition has been identified. Certain primer systems 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 (down to 0.2X of the original primer concentration) should be performed. The concentration of all primers should be adjusted in unison, preferably by using varying amounts of a stock of the primer mix.

Increasing primer concentration will generally lead to increased background amplification and is therefore not recommended. Reducing the primer concentration may reduce sensitivity and reaction yield, or it may increase the time required to amplify your target.

Dye

It is recommended to use EvaGreen® dye, however, users are free to use any fluorescent dye suitable for use in real time PCR. However, in such case optimization to determine required concentration of dye to be used in LAMP reaction will be needed.

Dilution buffer

Preparation of target dilutions in 25 mM Tris (pH 8.0) usually helps in increasing sensitivity especially for RNA targets. When using Tris as dilution buffer, it is very important to adjust pH to 8.0 (± 0.1) and filter the solution. Prepared solution can be stored at room temperature, however for long-term storage and to avoid contamination, it is recommended that solution should be aliqouted and stored at 4 °C.

Examples: Agarose Gels of Target-Specific and Nonspecific LAMP Amplification:

Correct target-specific amplification.

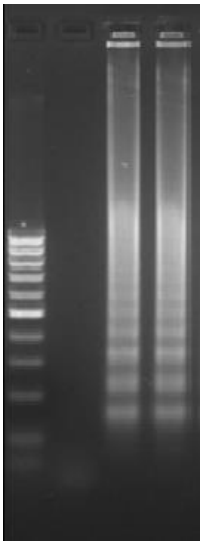


Figure 1: Lane 1: 100 bp Marker, Lane 2: Negative LAMP reaction. Lane 3 and Lane 4: Positive LAMP reaction products from an Positive Control template. A distinct banding pattern is seen among the smear.

Spurious background amplification.

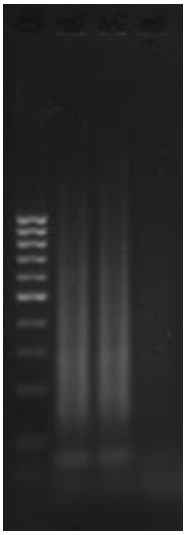


Figure 2: Lane 1: 100 bp ladder, Lane 2 and Lane 3: Background Amplification in a LAMP reaction. Non-specific or Background amplification appears as a single continuum of fragments with no visible or indistinct bands. A prominent primer dimer band is also characteristic of non-specific amplification. Lane 4: Negative LAMP reaction.

Additional Amplification Guidelines

Avoid Ribonuclease (RNAse) Contamination

Major sources of RNAse contamination in a typical laboratory include solutions and reagents, environmental exposure and contact with human hands and skin. Avoid introducing RNAses, rather than trying to remove them. Some basic precautions must be taken to work successfully with RNA.

- Always wear gloves to prevent introducing RNAse contamination from human hands.
- Change gloves frequently especially after touching skin, door knobs, and common surfaces.
- Use a set of pipettors dedicated solely for RNA work.
- Use RNAse-free plasticware and reagents.
- Designate an RNAse-free area of the lab.

Cold Reaction Set-Up

The OmniAmp polymerase has residual activity above 4 °C that can cause non-specific background amplification at temperatures below specific reaction temperature of 65 to 72 °C.

- All reactions using OmniAmp Polymerase 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.

Template Preparation

Most routine methods of template purification are sufficient (e.g. phenol/chloroform or guanidine/silica-based methods). However, trace amounts of purification agents (phenol, EDTA, Proteinase K, ethanol, etc.) may inhibit amplification. It is preferred that the nucleic template be dissolved in water or EDTA-free buffer rather than TE following purification. If TE is required, formulation with 0.1 mM EDTA will give best results.

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. This may slow the reaction.

dNTP's

For best results, or when sensitivity or reproducibility are critical, use a stock of dNTP's that have not undergone multiple freeze-thaws. LAMP systems can be more sensitive than PCR to the quality of dNTP's.

Dye for quantitation

If you intend to add a reagent for quantitation of the reaction or measurement of its progress, be aware that excessive dye may inhibit the reaction. Conditions will vary and will require optimization, but dye should be used at or below common working concentrations.

Appendix A: LAMP Resources Eiken PrimerExplorer Software: The Eiken PrimerExplorer software is an online software application that will assist users in designing a LAMP primer set. The software can be accessed at the URL listed below. For convenience, the user manual for this software has been published online as well.
PrimerExplorer link: http://primerexplorer.jp/elamp3.0.0/index.html.

Primer Explorer manual pages 1 through 16:
http://primerexplorer.jp/e/v3_manual/pdf/PrimerExplorerV3_Manual_1.pdf
Primer Explorer manual pages 17 through 37:
http://primerexplorer.jp/e/v3_manual/pdf/PrimerExplorerV3_Manual_2.pdf
Primer Explorer manual pages 38 through 37:
http://primerexplorer.jp/e/v3_manual/pdf/PrimerExplorerV3_Manual_3.pdf

Appendix B: Quality Control Assays

Activity Assay
Polymerase activity is assayed at 72 °C with 0.2 mM each of dATP, dGTP, dTTP, dCTP (mix of unlabeled and [^{33}P] dCTP); 10 µg activated calf thymus DNA, and 0.1 mg/mL BSA.

Absence of Endonuclease
OmniAmp Polymerase is determined to be free of detectable endonuclease or nicking activity. One µg of supercoiled plasmid DNA is incubated with enzyme for 16 hours at 70 °C. Agarose gel electrophoresis shows no alteration in mobility, consistent with endonuclease or nicking activity.

Absence of Exonuclease
OmniAmp Polymerase is tested to be free of contaminating exonuclease activity by incubating 1 µg of Hind III-digested lambda DNA with enzyme at 70 °C for 16 hours. Agarose gel electrophoresis shows no alteration in mobility, consistent with exonuclease activity.

Absence of Ribonuclease
OmniAmp Polymerase is tested to be free of contaminating RNAse activity by incubating with a fluorogenic RNAse substrate for 1 hour at 37 °C. No increase in assay fluorescence above background is detected.

Functional Assays
OmniAmp Isothermal Amplification system is tested for performance by isothermal amplification of regions of the MS2 bacteriophage RNA genome and the *E. coli* DNA genome. The resulting amplification products are visualized on ethidium bromide-stained agarose gels.

Appendix C: References

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.

Mori Y, Hirano T, Notomi T. Sequence specific visual detection of LAMP reactions by addition of cationic polymers. BMC Biotechnol. 2006 6:3.

Tomita N, Mori Y, Kanda H, Notomi T. Loop-mediated isothermal amplification (LAMP) of gene sequences and simple visual detection of products. Nat Protoc. 2008 3(5):877-82.

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