MASTISOPLEX®
DNA Lyo

Mast Group

MAST ISOPLEX®*DNA Lyo* kit

Frequently asked Questions and Answers

What is LAMP?

Loop-mediated isothermal amplification (LAMP), a nucleic acid amplification method developed in 2000, combines speed, simplicity, and high specificity. Several research and diagnostic tests have been developed based on this procedure. LAMP assays are performed under isothermal conditions using a strand displacement reaction, which employs a DNA polymerase and a set of six specially designed primers that recognize a total of eight distinct sequences on the target DNA.

What are the stages of the LAMP reaction?

The amplification cycle comprises two principal stages:
1. Copying of the target gene of interest using the specially designed gene primers to form the characteristic dumbell structure which is the starting point for the LAMP reaction. The formation of the dumbells is a continuous process, working simultaneously with the amplification reaction.
2. Isothermal amplification builds upon the dumbell starting unit to form long strands of alternately inverted repeats of the target molecular sequence on the same strand.

What is the difference between LAMP and PCR?

The isothermal reaction conditions of a LAMP assay allow for the continuous amplification of target genes, rather than being restricted to short periods of amplification as seen in polymerase chain reaction (PCR) cycling, which relies upon thermal cycling to achieve DNA strand separation, primer annealing and finally target extension. The continuous amplification of LAMP allows for the generation of much larger quantities of nucleic acids in a much shorter period of time. Production of huge quantities of nucleic acids (10^9 –10^{10} copies) have been reported within 15-60 minutes depending on the target and primer set.

What are the main design considerations for a new LAMP primer set?

Melting temperature (T_m)

In designing FIP (F1c and F2) and BIP (B1c and B2) primers

- F2 and B2 region – optimal sequence T_m ~ 60-65 ^oC, the optimal temperature for DNA polymerase.
- F1c and B1c region - sequence T_m values should be slightly higher than F2 or B2 in order that a looped out structure form immediately after release of the ssDNA from the template.

In designing F3 and B3 primers

- F3 and B3 region – T_m values must be lower than those of F2/B2 to ensure that synthesis occurs earlier from the inner primers than from the outer primers
- This is also ensured through excess addition of inner primers compared to outer primers (4 to 10-fold greater).

Amplicon Length

Length of target DNA important for LAMP efficiency as strand displacement DNA synthesis can be rate limiting.

- Best results obtained when target DNA (length between start of F2 and start of B2c) was between 130-200 bp.
- F2 to F3/B2 to B3 – optimum distance between primers is 0-20 bp.

GC Content

Design your assay so that the GC content of your primers is 35–65%, with an ideal content of 50%, which allows complexity while still maintaining a unique sequence. Primer sequences should not contain regions of 4 or more consecutive G residues.

Loop Primers

Increasing the size of your LAMP primer set from 4 to 6 by including loop primers (LoopF and LoopB) has been shown to improve sensitivity and reduce time-to-positive in LAMP reactions.

Complementarity and secondary structure:

Primers should be screened for self-dimers, hetero-dimers and hairpins structures

Specificity

Run a BLAST alignment to determine if the in-house designed primers are unique to the desired target sequence.

What are the optimal concentrations of LAMP primer sets in LAMP reactions ?

Primer Use recommendations:

Recommended primer concentration per 10µl LAMP assay for in-house primer sets are:

F3 and B3 – 2.5pmol

FIP and BIP – 20pmol

LoopF and LoopB – 10pmol

If initial results with this primer concentration are found to be unsuitable (i.e. the presence of false amplification in no template control reactions), we then suggest using half the concentration of primers in subsequent tests.

Where can I get help in designing a new LAMP primer set?

We recommend using Primer Explorer or LAMP Designer software as a resource in helping customers design their own LAMP primer sets for in-house tests.

Do LAMP primer sets need specific purification protocols?

Not necessarily. Standard desalted oligonucleotide primers are perfectly suitable for use in LAMP assays. HPLC purified primers will be of greater purity but will cost much more to synthesise.

Can I use standard PCR tubes to set up 10µl LAMP assays?

Yes, generic PCR tubes will work with MAST ISOPLEX®*DNA Lyo* reagents.

What equipment is needed to amplify MAST ISOPLEX®*DNA Lyo* reagents via LAMP assay?

An instrument capable of isothermal incubation of reaction tubes at the desired temperature (60-65^{o}C) such as the Applied Biosystems (ABI) 7500 FAST REAL-TIME PCR system, the ESEQuant TS system or equivalent in-house thermocycler. The equipment should have a fluorescent reader with FAM detection channel for recognition of amplification products

How many reactions should I perform to validate my primers of interest?

Positive and negative reactions for each primer set tested should be ideally performed in at least triplicate reactions to determine validity and consistency of result. Positive control primers and Sample

DNA tests should also be performed in tandem with in-house primer design tests to ensure reagents are performing accurately. Positive control primer and Sample DNA tests, in tandem with no-template control (NTC) should also be ideally performed in triplicate. No primer control (NPC) reactions should also be considered as optional extra tests but are less important than NTC assays.

Once assays are proven, a single positive and negative control reaction per run is suggested.

What is the shelf life?

MAST ISOPLEX®*DNA Lyo* reagents have a shelf life of one year if stored at room temperature (18°C to 25°C) away from direct sunlight.

How should I store these kits?

Store the unopened kit at room temperature (18°C to 25°C) away from direct sunlight until the expiry date shown on the pack label. Once reconstituted, MAST ISOPLEX®*DNA Lyo* pellets, Positive Control Primers and Positive Control DNA should be aliquoted and stored at -20°C. All other pack components should remain stored at room temperature away from direct sunlight. Reconstituted MAST ISOPLEX®*DNA Lyo* pellets should be used up within 8 weeks after first resuspending the vial.

How many times can I freeze thaw MAST ISOPLEX®*DNALyo* reagents once reconstituted?

Reconstituted MAST ISOPLEX®*DNA Lyo* pellets should not be freeze thawed more than 5 times. Reconstituted MAST ISOPLEX®*DNA Lyo* Positive Control DNA and Positive Control Primers should not be freeze thawed more than 5 times either (keep in small aliquots to prevent this occurrence).

Do MAST ISOPLEX®*DNA Lyo* reagents maintain activity if exposed to different temperatures and humidities?

Yes, MAST ISOPLEX®*DNA Lyo* reagents, when lyophilised and sealed in their protective aluminium foil pouches with pre-packed desiccant, can be exposed to temperatures as high as 50°C and relative humidity over 90% for 8 weeks without any impact on reagent efficiency.

Similarly, lyophilised reagents can be stored at temperatures as low 4°C for the duration of the product's shelf life without loss of activity.

What DNA preparation method is needed?

Although LAMP reagents are traditionally less susceptible to inhibitors compared to standard PCR components, we recommend purifying target DNA of interest to obtain suitable specificity and sensitivity data. However, crude extractions from bacterial cultures (bacteria grown on Columbia agar, lysed directly in 0.85% saline and heated to 95^{o}C for 5 minutes) have been shown to work extremely well when 1µl of this is added directly per 10µl assay to resuspended MAST ISOPLEX®DNA Lyo pellets, amplifying target DNA as sensitively and specifically as purified extracts.

What does a positive result look like?

If your target is present, large quantities of dsDNA products will be produced during the LAMP reaction. The dsDNA intercalating dye present in the MAST ISOPLEX®*DNA Lyo* reagents will bind to these products, generating an increased fluorescence signal. Thus, positive amplification graphs on thermocyclers such as the ABI 7500 FAST REAL-TIME PCR system typically show an 'S' shaped sigmoid curve, indicating the increase in fluorescence observed on detection of positive sample DNA.

How fast should I expect a result?

The speed of target amplification depends on a number of factors including primer design, purity of target DNA and the quantity of target DNA added as input into the LAMP reaction. Positive results can be obtained with MAST ISOPLEX®*DNA Lyo* reagents between 5 and 40 minutes.

What is the optimal temperature for running LAMP reactions?

MAST recommends using MAST ISOPLEX®*DNA Lyo* reagents in LAMP reactions at 63°C, although LAMP reactions can be tested within a range of 60–65°C if required for optimisation purposes.

What is the optimal concentration of reconstitution buffer for use in LAMP reactions ?

The recommended final concentration of reconstitution buffer in each 10µl LAMP reaction is 0.1M Tris (RB comes at 0.5M concentration in the MAST ISOPLEX®*DNA Lyo* kit).

How can I ensure the MAST ISOPLEX®*DNA Lyo* kit I bought is of suitable quality?

It is recommended that quality control on MAST ISOPLEX®*DNA Lyo* pellets is performed by the customer using the Positive Control DNA, the Positive Control Primer, Reconstitution Buffer and the molecular grade water supplied in the kit per test run. These tests will ensure that reagents are performing as specified and no contamination of kit reagents has occurred. If control reactions are incorrect, check for signs of deterioration or contamination of kit reagents and perform a retest. If retest also fails, contact Mast technical support department for advice.

Do I need to run control reactions?

We recommend running control reactions, both positive and negative, in tandem with all tests performed using MAST ISOPLEX®*DNA Lyo* reagents to validate all components and reaction conditions.

What precautions do I need to be aware of while using a MAST ISOPLEX® *DNA Lyo* kit ?

Despite the fact that MAST ISOPLEX® *DNA Lyo* kits do not contain any biological or chemical hazards, they are designed to be used by trained laboratory personnel only.

During LAMP assay set-up, reaction tubes should be kept closed at all times following addition of reagents and discarded without opening following use, according to local health and safety guidelines.

To avoid any contamination with the amplified product, never open a vial after amplification.

Do not vortex reaction tubes. Ensure all reaction tubes are not scratched or cracked prior to use.

Are the time-to-positive results related to the amount of amplified product?

Yes. MAST ISOPLEX® *DNA Lyo* pellets contain a fluorescent DNA-intercalating dye which directly binds to amplification products. Thus, the faster that amplification occurs in a sample, the faster a fluorescent signal becomes apparent on the thermocycler's FAM channel because of the dye's interaction with these newly-synthesised DNA products.

Can I use amplification reactions for the quantification of input DNA?

Unlike qPCR, LAMP reactions do not amplify DNA exponentially. As such, target DNA concentrations can be estimated but not quantified using LAMP.

How can I eliminate/reduce nonspecific or no-template amplification?

Isothermal amplification reactions can be extremely rapid and robust, but commonly exhibit nonspecific amplification. This can show up as false positives and must be controlled for accurate interpretation of results. Although changes to assay temperature and reconstitution buffer concentration can improve assay reliability, thorough optimization of each newly-designed LAMP primer set is typically the most effective method for ensuring LAMP specificity and sensitivity. There are many different parameters to consider in primer design such as length, GC content, location, melting temperature, all of which can play a key role in assay success (see primer design question for further details). Also, the quantity of each primer added per reaction is crucial and must be optimized, as well as the concentration ratio between primers types (between inner, outer and loop primers).

Do I need to do a pre-heat before amplification?

No, a 95°C pre-heat incubation of MAST ISOPLEX®*DNA Lyo* reagents should not be performed as this temperature would damage components of the MAST ISOPLEX®*DNA Lyo* pellet and destroy assay efficiency. Denaturation of in-house designed LAMP primer sets and/or template DNA at 95°C for 5 minutes may be performed separately before addition to MAST ISOPLEX®*DNA Lyo* reagents if required for assay optimisation purposes but this is entirely optional.

How do you set up the assay if DNA sample volume needs to be greater than 1µl/reaction?

If DNA sample volume needs to be greater than 1µl/reaction, the pellet can be resuspended in an appropriate quantity of reconstitution buffer and water to bring the concentration of the assay to optimum guidelines. Using a smaller volume of water during reconstitution is the most effective way of doing this.

Example: if using 1µl of sample/LAMP reaction, a MAST ISOPLEX®*DNA Lyo* or *DNA Lyo Plus* pellet can be resuspended in 20µl of reconstitution buffer (RB) alongside 68µl of molecular grade water (WTR).Ten 10µl LAMP reactions can then be generated using this 88µl mixture, each consisting of 8.6µl reconstitution mix, 0.2 µl of primers and 1µl of sample. The final LAMP assay concentration in these tests would be 0.1M Tris. If 2µl of DNA sample is required per reaction, the most effective way of incorporating this extra volume of sample in a 10µl LAMP reaction is to reduce the volume of water in the reconstitution mix. MAST ISOPLEX®*DNA Lyo* or *DNA Lyo Plus* pellets would be resuspended in 20µl of reconstitution buffer (RB) alongside 58µl of molecular grade water (WTR). This 78µl mixture could be used in ten reactions at volumes of 7.8µl reconstitution mix per reaction, run alongside 0.2 µl of primers and 2µl of sample. Thus these LAMP reactions, while incorporating more sample DNA, would maintain the optimal final assay concentration of 0.1M Tris.

How could I check that an amplification curve in an unknown sample is specific for a target of interest?

In this instance, it may be informative to run a melt curve on your thermocycler after the LAMP assay has been completed. If the unknown sample displays a T_m (melting temperature) at the same or similar temperature as samples known to contain the target of interest, this suggests that targets of the same sequence are present in the unknown sample.

Melt curve analysis is also instructive in determining if amplification curves seen in no-template control (NTC) reactions are a product of contamination of the sample or simply due to primer dimerization.

Is the MAST ISOPLEX® *DNA Lyo* kit compatible for DNA targets only?

MAST ISOPLEX®*DNA Lyo* kits can amplify DNA but not RNA as their formulation contains Bst Polymerase but not Reverse Transcriptase. However, the addition of externally-purchased Reverse Transcriptase to reconstituted MAST ISOPLEX®*DNA Lyo* pellets would allow target RNA to be amplified, depending on how well the target RNA LAMP assay was optimised by an end-user.

Can I still use this kit if I haven't got access to a real time thermocycler?

MAST ISOPLEX®*DNA Lyo* kits have been primarily adapted for use in real-time analysers with a FAM detection channel. If no such equipment is available, these reactions can still be run in real-time turbidimeters.

If no-real time analysis is required and simply determining the presence/absence of amplification products is the main aim of an experiment, LAMP assays using MAST ISOPLEX®*DNA Lyo* reagents can be run in hot blocks or end point thermal cyclers-set between 60 to 65^{o}C. Once incubation is complete, DNA gel loading dye (purchased separately) can be added to the amplified product and loaded onto an agarose gel for electrophoresis. Positive amplification products from these reactions would be indicated by the presence of several ladder-like DNA fragments. Negative control lanes would either be completely empty of product or contain low-molecular weight bands at the bottom of the gel indicating primer dimers.

If using MAST ISOPLEX®*DNA Lyo* reagents for turbidimeter or gel electrophoresis tests, optimisation of the entire assay process would need to be performed by the end user to determine the validity of results.