SynLAMP Mix

MasterMix for special PCR reactions

CAUTION! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from synthbioenzymes.com.

The protocols and storage conditions detailed below ensure that the SynLAMP Mix can function at its highest potential. These guidelines cover standard amplification protocols for all LAMP reactions and unique specifications tested to provide optimal conditions for SynLAMP Mix.

1. Product description

SynLAMP Mix is the perfect choice to easily and effectively shorten special PCR reaction times like isothermal amplification (LAMP), whole genome amplification (WGA) or multiple displacement amplification (MDA). SynLAMP Mix works efficiently with multiple amplification inhibitory factors such as high GC content, secondary structures in template, and low template concentration. It includes all of the necessary components for the reaction, such as the buffer, enzymes, dye, dNTP in a single, pre-mixed solution. This makes it much easier and more efficient to set up experiments, as you don't have to measure out each component every time you perform the reaction. The dye in the enzyme is an intercalating dye that can be used to track a LAMP reaction in real-time by fluorescence measurement. One of the enzymes is the SynBst that is the engineered large fragment of *Bacillus stearothermophilus* DNA polymerase, it has robust strand displacement activity and catalyses the 5'-3' synthesis of DNA without detectable 3′-5′ exonuclease activity. The other enzyme, SynRT has a very effective reverse transcription activity, is capable to highly efficient full-length cDNA synthesis in a wide range of sample types up to 12 kb and does not contain RNaseH.

2. Contents

SynLAMP Mix package information

L011M	100 x 25 µl reactions Material provided: 2x SynLAMP Mix (1250 µl), 100 mM $MgSO_4$ solution (1.5 ml) and 50x LAMP Fluorescent Dye (50 µl)
L011L	500 x 25 µl reactions Material provided: 2x SynLAMP Mix (5x 1250 µl), 100 mM $MgSO_4$ solution (5 x 1.5 ml) and 50x LAMP Fluorescent Dye (250 µl)

3. Storage conditions for SynLAMP Mix

Although our experiments confirm that the SynLAMP Mix is viable after incubation at room temperature or 4°C for 2 weeks, we recommend storing all components at -20°C to ensure its quality.

4. Guidelines for using SynBst Polymerase

All reaction preparations should be done over ice with sterile lab equipment. All components of the reaction mix should be mixed and centrifuged gently.

4.1 Standard LAMP reaction protocol

Primers

We recommend using online programs to design primers suitable for the LAMP reaction. These programs analyze the desired template and determine the optimal length, GC content and melting temperature. We recommend PrimerExplorer for Loop-mediated isothermal amplification (LAMP) and Primer3web for other isothermal amplification-based reactions like whole genome amplification. Ordering oligonucleotides in different purification can increase the efficiency of the LAMP reaction, but experiments show that the standard desalted primers suffice.

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It is important to make a primer mastermix for the reaction, in which the concentration of each primer should be the following (for standard LAMP reaction):

Component	10x concentration (stock)	1X concentration (final)
FIP Primer (Forward inner primer)	16 µM	1.6 µM
BIP Primer (Backward inner primer)	16 µM	1.6 µM
F3 Primer (Forward outer primer)	2 µM	0.2 µM
B3 Primer (Backward outer primer)	2 µM	0.2 µM
LoopF Primer	4 µM	0.4 µM
LoopB Primer	4 µM	0.4 µM

The recommended reaction protocol for the SynLAMP Mix is the following.

Component	DNA/RNA target detection
SynLAMP Mix (2x)	12.5 µl
LAMP Primer Mix	2.5 µl
DNA / RNA sample (>10 copies)	variable
Fluorescent Dye (50x)	0.5 µl
H2O	to 25 µl

Incubate the reaction mixture at 65°C for 30-60 minutes. We recommend fluorescent detection every 30 seconds.

⚠ NOTE! The SYBR®/FAM channel of common real-time fluorimeters can be used to read the dye.

4.2 Additives

Additives

Although several additive substances were tested which can improve the amplification time of the LAMP reaction, - the most commonly used being the Guanidine hydrochloride -, the SynBst Polymerase have been engineered to be more effective than the general Bst polymerases with guanidine hydrochloride added to their reaction mixture. As the SynBst polymerase is more optimized than other Bst Polymerases we **DO NOT** recommend using Guanidine hydrochloride as an additive substance as it will not improve the amplification time.

The concentration of magnesium is important for the LAMP reaction to take place, as it is the cofactor of the polymerase enzyme. Therefore, the concentration of Mg^{2+} significantly affects the activity of the enzyme and the PCR reaction. If optimization is desired raise the final Mg^{2+} concentration within the 4–10 mM range. Note that excess Mg^{2+} can also stabilize spurious annealing of primers to incorrect template sites and decrease specificity.

5. Troubleshooting

Visit our online FAQ for tips and tricks and troubleshooting information:

https://synthbioenzymes.com/product/synlamp/