A&A BIOTECHNOLOGY
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Manual

BeST™ LAMP Kit SYBR®

Kit for isothermal DNA amplification in constant temperature with SYBR® Green.

catalog #	size
1025-100G	100 reactions in 25 µl

For research use only.

Guarantee

A&A Biotechnology provides guarantee on this product.
The company does not guarantee correct performance of this kit in the event of:

- not adhering to the supplied protocol
- use of not recommended equipment or materials
- use of other reagents than recommended or which are not a component of the product
- use of expired or improperly stored product or its components

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Description

LAMP (Loop Mediated Isothermal **AMP**lification) is an isothermal nucleic acid amplification technique. In contrast to polymerase chain reaction (PCR) technology, which consists of repeated steps carried out in different temperatures, isothermal amplification is performed at a constant temperature and does not require a thermal cycler.

Contents

	1025-100G	**storage**
Green BeST™ reaction mix	1 x 1.40 ml	-20 °C
BeST™ polymerase	1 x 120 µl	-20 °C
positive control λ phage DNA template λ primer mix	1 x 70 µl	-20 °C
ultrapure water	1 x 1.5 ml	-20 °C

Notes

- The reaction should be prepared on ice and tubes with reaction mix should be put immediately into a thermocycler or heating block preheated to temp. 65 °C.
- A&A Biotechnology offers free help in designing primers for LAMP reaction as well as substantial help.

Additional equipment and reagents

- LAMP primers mix
- Ultrapure water for preparation of LAMP primers mix and DNA template suspension (cat. # 005-515, 005-1015, 005-2515, 005-5)
- TE buffer for DNA template suspension (cat. # K-TE-5, K-TE-100)

Advantages of LAMP technique

1. It allows for efficient and highly specific amplification of DNA by using three pairs of primers complementary to a suitable sequence of targeting DNA. Due to the specificity of primers the quantity of amplified DNA in the reaction is also considerably higher than in the PCR.
2. Fast amplification of DNA enables quick and easy identification of human, animal and plant pathogens - **detection time from 30 min up to 1.5 hours** is dependent on the quality and concentration of the target DNA. In case of phage lambda DNA with a concentration range between 5-50 ng/µl the amplification time of the DNA sequence is less than 30 min.
3. BeST™ polymerase used in the LAMP reaction is a modified version of *Bst* polymerase from *Bacillus stearothermophilus*, lacking the 5'-3' exonuclease domain. In addition to a replication activity BeST™ polymerase has high strand displacement activity which eliminates denaturation of the DNA template.
4. The amplified products are concatemers of different sizes forming a ladder-pattern structure which could be detected by agarose gel electrophoresis or by monitoring the amplification in real time using fluorescent dye binding to double-stranded DNA or dyes sensitive to pH changes.

Reaction setup

Before starting the reaction:

1. LAMP primers mix

The LAMP primers mix is prepared with all 6 primers. The LAMP primers mix should contain:
40 µM FIP, 40 µM BIP, 5 µM F3, 5 µM B3, 10 µM LoopF, 10 µM LoopB - each suspended in water.

LAMP primers mix can be stored at -20 °C until later use.
Primers desalted by precipitation are sufficient to carry out the LAMP reaction.

The example of preparing the LAMP primers mix:

primer [100 µM]	FIB	BIP	F3	B3	Loop F	Loop B	water
volume [µl]	10	10	1.25	1.25	2.5	2.5	122.5

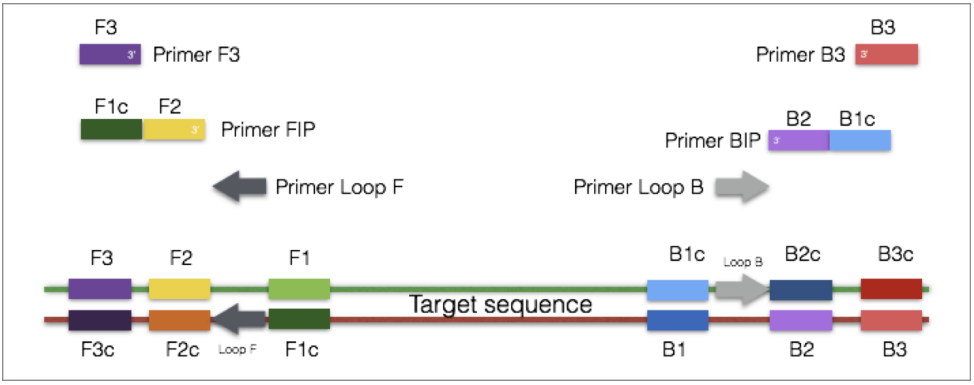

Fig. 1 Scheme of the LAMP primers system.

2. DNA template

DNA template should be suspended in water or TE buffer, pH 8.0 (not included).

Setting the LAMP reaction

Attention!!

The reaction should be prepared on ice and tubes with reaction mix should be put immediately into a thermocycler or heating block preheated to temp. 65 °C.

1. Experimental reaction

component	volume
Green BeST™ reaction mix	12.5 µl
DNA template [0.01-500 ng]	1 µl
LAMP primers mix	3 µl
BeST™ polymerase	1 µl
ultrapure water	up to 25 µl

Gently mix by vortexing and briefly centrifuged.

2. Control reaction (ג phage DNA template)

component	volume
Green BeST™ reaction mix	12.5 µl
positive control	4 µl
BeST™ polymerase	1 µl
ultrapure water	up to 25 µl

Gently mix by vortexing and briefly centrifuged.

3. Isothermal amplification conditions

step	temperature	time	cycle
* LAMP reaction - the fluorescence read after each minute (fig. 2)	65 °C	1 min	30
** the example program for the melting curve (fig. 3)	95 °C	1 min	
	60-95 °C gradient		
	95 °C	30 s	

* In case of first use of the kit it is recommended to optimize the reaction by extending the first step to 60 cycles.

** Additionally, the melting curve measurement can be set up to ensure that there are no nonspecific products after the LAMP reaction. Melting curve analysis is based on gradually raising the temperature of the mixture by 0.5 °C and measuring the fluorescence until DNA denaturation. Once the melting point of the product has been reached it is followed by a very rapid denaturation and a sharp decline in fluorescence. If there is only one specific product there is one peak because each strand of DNA has only one characteristic melting point (fig. 3).

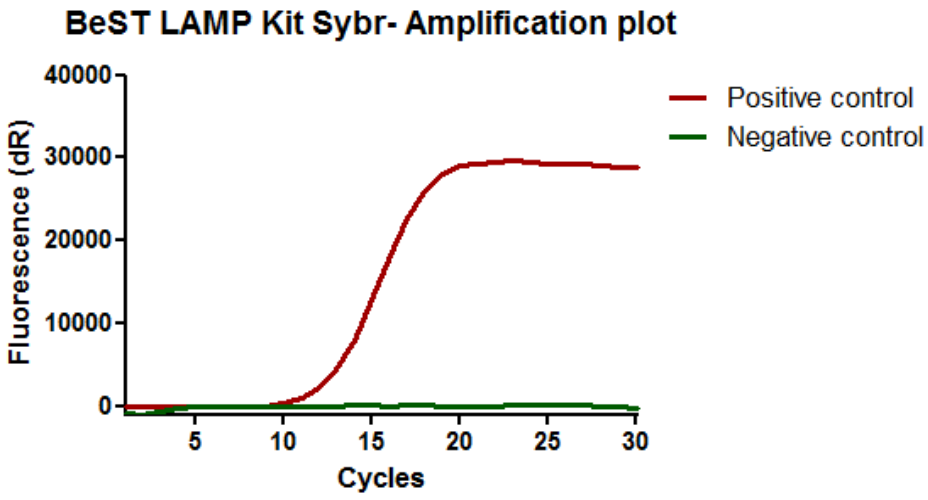

Fig. 2 The curve of amplification during the LAMP reaction.
positive control - LAMP reaction on the λ phage DNA matrix.
negative control - LAMP reaction without DNA template.

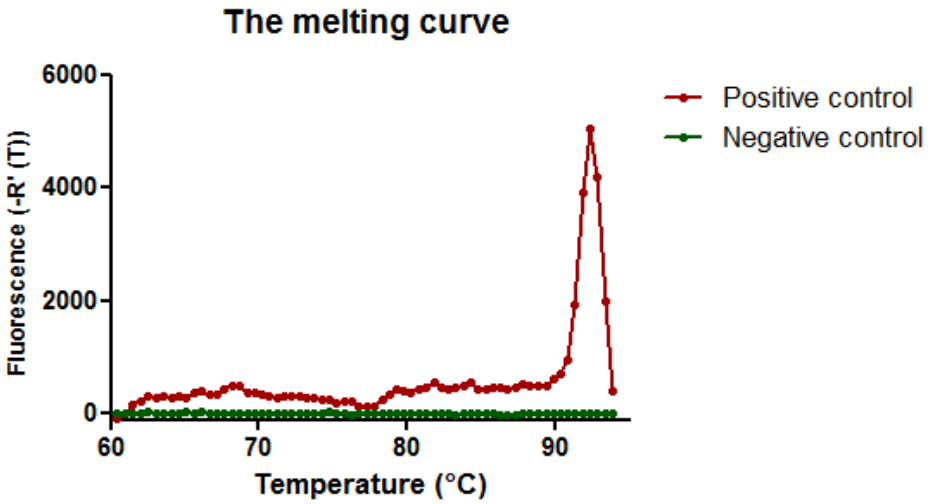

Fig. 3 The melting curve during the LAMP reaction.
positive control - LAMP reaction on the λ phage DNA matrix.
negative control - LAMP reaction without DNA template.

Detection of LAMP reaction

The DNA analysis after the LAMP reaction should be carried out by electrophoresis agarose gel. We recommend electrophoresis on 1.5% agarose gel with ethidium bromide to the degree of separation indicated on Fig. 4..

Fig. 4 Detection of LAMP products by agarose gel electrophoresis stained by EB (ethidium bromide).
K+ LAMP reaction with λ phage DNA
K- LAMP reaction without DNA template

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