Bst DNA polymerase Kit

Cat# no.: A9008

Store at -20ºC

For Research use Only

1.1 Ordering Information

Kit components	A9008-10	A9008-25	A9008-50	A9008-100
***Bst* DNA Polymerase**	10µL	25µL	50µL	100µL
10X LAMP Reaction buffer	25µL	62.5µL	125µL	250µL
dNTPs (10mM)	35µL	87.5µL	175µL	350µL
100mM $MgSO_4$	15µL	37.5µL	75µL	150µL
Nuclease-free water	500µL	1mL	1.5ml	1mL x 2

1.2 Introduction

Nucleic acid amplification is one of the most valuable tools in all life sciences fields, in diagnosis of various diseases. Bst DNA polymerase uses strand displacement amplification, eliminating the varying thermal steps the sample is subjected to, isothermally amplifying the target sequences with specificity, efficiency and speed. It is a simple process that can be done even on a heat block or water bath without any expensive equipment making it cost-effective.

1.3 Description

ProEnz Bst DNA polymerase of approximately 90 kDa, purified from the expression product of E. coli strain carrying the gene from Bacillus stearothermophilus. The enzyme catalyzes the polymerization of nucleotides by Loop-Mediated Isothermal Amplification (LAMP). This property allows primer annealing and subsequent amplicon elongation without the need for heat denaturation of dsDNA.

Bst has 5' to 3' polymerase and exonuclease activity. It lacks 3' to 5' exonuclease activity.

LAMP reaction requires a set of 4-6 primers which recognize 6-8 distinct regions of target DNA.

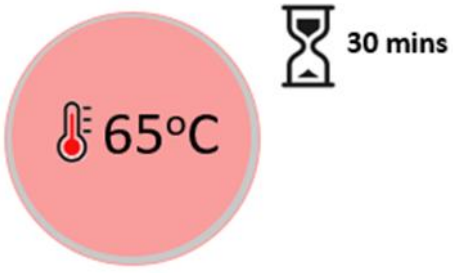
1.4 Salient features

- Possesses proof-reading 5'-3' exonuclease activity.
- Strand displacement synthesis
- High specificity
- Cost-effective

1.5 Principle of the technique

In LAMP, four primers are used in the initial steps which generate a stem-loop DNA for subsequent LAMP cycling during which the target region is recognized by four sequences. And subsequently two primers, to ensure high-specific target amplification.

1.6 LAMP steps/conditions



1.8 Applications

1. For amplification by Loop Mediated Isothermal Amplification (LAMP)

2. For highly efficient, accurate and sensitive analysis of infectious or genetic diseases.

1.9 Storage & handling

Store all the components at -20 °C and make sure to thaw on ice prior use.

1.10 Protocol

1. Place all the components on ice for thawing 20-30mins prior.
2. After ensuring that all the components are thawed completely, gently vortex the components and give a brief spin and place the vials on ice.
3. A LAMP Primer mix can be prepared with all 4 or 6 (with Loop) primers. A 10X Primer Mix should contain: 16 µM FIP, 16 µM BIP, 2 µM F3, 2 µM BE, 4 µM LoopF, 4 µM LoopB in TE or water.
4. Place 1.5mL microcentrifuge tubes, for the required number of reactions, on ice and add the components for 25µl reaction volume as given in Table-1.

Table-1:

Components	Concentration	Vol. for 25 µL
Bst DNA polymerase	8U/µL	1 µL
10x LAMP Reaction buffer	1X	2.5 µL
FIP/BIP Primers mix (25X)	1X	1 µL
F3/B3 Primers mix (25X)	1X	1 µL
Loop F/B Primers mix(25X)	1X	1 µL
dNTP (10mM each)	1.4mM- 6mM	3.5 µL
Template	-	Variable
Nuclease free water	-	Make up to 25 µL

5. It is recommended to add the enzyme as the last component to the tube.
6. After ensuring that all components have been added to the tube, spin the tubes in a microcentrifuge and ensure no bubbles are visible in the tubes.
7. Place these vials in a heat block or water bath with temperature set to 65ºC for 30mins.
9. Note the end time.
10. In the meantime, prepare an agarose gel of desired concentration. After solidification, submerge the gel in 1X TAE/TBE buffer.
11. After the Isothermal amplification, take out the tubes and add DNA loading dye to the tubes and load the samples on the gel and run till the dye front can be seen 3/4th the gel.
12. Observe the gel under UV light for desired bands.

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1.11 Unit definition

One unit is the amount of enzyme that will incorporate 10 nmol of dNTP into acid- insoluble products in 30 minutes at 65°C.

1.12 Must follow

Store enzyme and buffer at -20°C.
If turbidity/precipitation is observed in buffer and enzyme, discard the vials immediately.

1.13 10X LAMP reaction buffer composition

200mM Tris-HCl (pH 8.8), 100mM $(NH_4)_2SO_4$, 100mM KCl, 20mM $MgSO_4$, 0.1% Triton X-100.

1.15 Materials required but not supplied

1. Template
2. 0.2ml PCR tubes
3. Primers
4. Pipette tips
5. Agarose
6. 1X TAE/TBE buffer
7. DNA loading dye
8. DNA ladder

1.16 Limitations

1. The major disadvantage is the designing of the primers. Any small error in primer designing can lead non-specific amplification or no amplification.
2. This makes it difficult for no-specialists.
3. Prone to contamination.
4. Using a separate area to open tubes post reaction is recommended.

1.17 Optimal results can be obtained by:

1. Clean the working bench with 70% ethanol or isopropyl alcohol before placing anything on the bench.
2. Wear a fresh pair of protective nitrile powder-free gloves before setting up the reaction.
3. Let all the components thaw on ice completely prior to vortex them.
4. Avoid repeated freeze-thaw cycles.
5. To avoid repeated freeze-thaw cycles, aliquot enzyme and buffer with volume required for single use.
6. Use properly calibrated pipettes.

Explanation of symbols

REF -Catalogue number

- Expiry

LOT - Lot number/Batch number

-25°C max
-15°C min
- Storage limitations

-Manufacturing: Plot No. 147/D, Phase II, Cherlapally IDA, Telangana, Hyderabad - 500051, India.

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