TIARIS™ LAMP BstI DNA polymerase Kit

(Molecular Biology Grade)

Ordering info

TBK0082, 40 U

TBK0083, 1.600 U

TBK0084, 3.200 U

Description

TIARIS™ LAMP BstI DNA polymerase Kit is a convenient kit to amplify . It includes a mesophilic polymerase from *Geobacillus stearothermophilus*. Large fragment of BstI polymerase is produced in a recombinant way in *Escherichia coli*. The enzyme allows a rapid and efficient amplification by its strong displacement activity. The kit is useful to amplify minimally purified samples.

Features

- Without exonuclease activity.
- Optimal activity at 60-65°C.
- Inactivated at 80°C.
- Highly sensitivity, amplification factor of up to 10^9.
- Flexible readout.

Applications

- Suitable to nucleic acid amplification of minimally processed samples.
- Nucleic acid amplification by LAMP or RT-LAMP.
- Strand displacement.
- DNA sequencing.
- Whole genome amplification.
- Point of care (POC) or *in situ* detection systems.

Kit Components

Components	TBK0083	TBK0084
BstI DNA polymerase (8 U/ µL)	200 µL	400 µL
BstI Buffer (10x)	2 x 0.5 mL	6 x 0.5 mL
$MgSO_4$ (0.1 M)	1.5 mL	1.5 mL
High-Q™ dNTPs Mix 10 mM TOTAL	1 mL	2 mL

Order Info Kit Components: *BstI DNA polymerase (TBZ0214) | BstI Buffer 10x (TBB0315) | $MgSO_4$ 0.1 M (TBR0222) | High-Q™ dNTPs Mix 10 mM TOTAL (TBR0209).*

Storage

Store at -20°C.

The product is shipped on blue ice.

Unit Definition

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

Quality Control

Specific activity tested.

Purity determined by SDS-PAGE: >99%.

DNAse and RNAse activity not detected.

Material required (*not included*)

- PCR Grade Water (TBB0303)
- Specific Primers
- SYBR

PROTOCOL

To optimize the reaction conditions use this final concentration ranges: BstI polymerase (0.04–0.32 U/µL), $MgSO_4$ (4-10 mM). Temperature range between 50–68°C could be evaluated.

1. Prepare Primer Stock Solution (10x)

Primer	Stock Concentration	Final Concentration	Volume
F3, *forward outer primer*	100 pmol/ µL	2 pmol/ µL	2 µL
B3, *forward outer primer*	100 pmol/ µL	2 pmol/ µL	2 µL
FIP, *forward inner primer*	100 pmol/ µL	8-16 pmol/ µL	8-16 µL
BIP, *reverse inner primer*	100 pmol/ µL	8-16 pmol/ µL	8-16 µL
LF, *forward loop primer*	100 pmol/ µL	4-10 pmol/ µL	4-10 µL
LB, *reverse loop primer*	100 pmol/ µL	4-10 pmol/ µL	4-10 µL
PCR Grade Water			up 100 µL
Total Volume			100 µL

2. Thawing all components on ice. Vortex and spin them.

3. On ice, prepare a mix of the following components,

Reaction Components	Final Concentration	Volume
BstI Buffer (10x)	1x	2,5 µL
$MgSO_4$ (0.1M)	6 mM*	1,5 µL
High-Q™ dNTP Mix 10 mM TOTAL	400 µM TOTAL	1 µL
Primer Stock Solution (10x)	1x	2,5 µL
BstI DNA polymerase (8U/ µL)	0,32 U/ µL	1 µL
DNA Sample	< 10 copies	X µL
PCR Grade Water		up 25 µL
Total Volume		25 µL

** Final Concentration is 8 mM due to 2 mM additional from BstI Buffer. Mg^{2+} optimal range is 6-8 mM.*

4. Incubate at 65°C for 30-60 minutes.

5. Reactions could be analyzed by agarose gel electrophoresis (*a series of concatamers of different sizes are observed*), turbidimetry, colorimetry by color change of added dyes, or by fluorescence detection (*including SYBR in the reaction at final concentration of 1-2 µM*). In SYBR presence, LAMP reaction could be analyzed by naked-eye visualization (*negative reaction remained orange and positive, turn green*), using a portable UV or monitoring with a fluorimeter.