User Guide

BST Max DNA Polymerase

Salt and Inhibitor Tolerant; Recombinant, from *E. coli*

SRE0113

Product Overview

Description

BST Max Isothermal DNA Polymerase is an engineered recombinant salt- and inhibitor-tolerant enzyme.

Applications for BST Max DNA Polymerase include: loop-mediated isothermal amplification (LAMP),[1] reverse transcription (RT) LAMP, and multiple displacement amplification (MDA).

The enzyme is supplied at 8U/uL.

Features

- Active from 25-65 °C (optimal at 65 °C)
- Optimal salt concentration 50-350 mM
- No 5′-3′ or 3′-5′ exonuclease activity
- Strong strand displacement activity

Unit Definition

One unit is the amount of enzyme required to incorporate 10 nmol of dNTP into acid insoluble material at 65 °C for 30 minutes.

Reagents Provided

- BST Max DNA Polymerase 8 units/µL in 10 mM Tris-HCl pH 7.5, 100 mM KCl, 0.005% Tween 20, 1 mM DTT, 0.1 mM EDTA, and 50% glycerol (SRE0113A)
- 10X BST Max Buffer (SRE0113B)
- 100 mM $MgSO_4$ (SRE0113C)

Materials and Reagents Required

(not included)

- Deoxynucleotide (dNTP) Mix, containing 10 mM each of dATP, dCTP, dGTP, and dTTP sodium salts (D7295)
- Nuclease-free water (W1754)
- Custom ordered primers specific to gene of interest (OLIGO)
- PCR tubes or plates
- Sample containing template DNA
- Thermal cycler, heat block, or incubator

Precautions and Disclaimer

This product is for R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store at –20 °C.

Suggested Protocol

Step	Description

Assemble Reaction Mix

For best reproducibility, assemble a master mix of PCR reagents by multiplying the number of reactions needed (plus 10% to account for pipetting error) by the suggested volumes in the table below.

Reagent	Final Concentration	Amount per 25 µL reaction
10X Reaction buffer	1X	2.5 µL
BST Max	8 U	1 µL
dNTP Mix, 10 mM	1.4 mM	3.5 µL
*10X Primer Mix	1X	2.5 µL
**$MgSO_4$	variable	variable
Intercalating dye (optional)	variable	variable
Nuclease-free Water	N/A	To 23 µL total

* A 10X Primer mix consists of 2 µM forward primer, 2 µM backward primer, 8 µM loop forward primer, 8 µM loop backward primer, 16 µM forward inner primer, and 16 µM backward inner primer. Primer concentrations may need to be optimized empirically.

** BST Max is a magnesium ion-dependent enzyme. Optimal concentrations of template DNA, primers, and $MgSO_4$ will be target-specific.

Add Template

Aliquot 2 µL (>4pg total) of DNA template into PCR plate on ice. Add chilled reaction mix to wells.

Amplify

Incubate reaction at 65 °C for 30 minutes (or up to 1 hour). Amplification can be monitored using a real time PCR instrument compatible with intercalating dye used in reaction setup. Alternatively, amplified product can be detected using various endpoint methods.[2]

Technical Considerations

- BST Max DNA Polymerase is most active at pH 8.5 in buffer supplemented with 4–20 mM $MgSO_4$ and 50–350 mM salt (NaCl or KCl) at 65 °C.
- Deactivation occurs when placing enzyme at 95 °C for 1 minute.
- Optimal concentration of $MgSO_4$ will be target-specific. The 1X BST Max buffer contains 4 mM $MgSO_4$. The concentration can be increased with the supplied $MgSO_4$ to improve sensitivity but should not exceed 8 mM as this may result in non-specific amplification. To optimize $MgSO_4$ concentration, follow the Suggested Protocol using a fixed template concentration and compare the following conditions side-by-side:

100mM $MgSO_4$ Added (µL)§	Final $MgSO_4$ Concentration (mM)
0	4
0.5	6
1	8

§ Based on a 25 µL final reaction volume

Include a no template control (NTC) to ensure absence of non-specific activity. Ideal $MgSO_4$ concentrations should maximize difference in time to result (e.g. cycle threshold value) between NTC and experimental sample.

Product Ordering

Order products online at SigmaAldrich.com.

Description	Catalogue Number
MMLV Reverse Transcriptase	M1302
GenElute™ Mammalian Genomic DNA Miniprep Kit	G1N10
GenElute™ PCR Clean-Up Kit	NA1020
GenElute™ Plant Genomic DNA Miniprep Kit	G2N70
GenElute™-E Single Spin DNA Cleanup Kit	EC600
Water, Microbial DNA-free	MBD0025
Nuclease-Free Water, for Molecular Biology	W1754

References

1. Notomi, T. et al., Nucleic Acids Res., 28, e63 (2000).
2. Fischbach, J. et al., BioTechniques., 58, e4 (2015).

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