Bst 2.0 WarmStart® DNA Polymerase

1-800-632-7799
info@neb.com
www.neb.com

M0538S 003141216121

M0538S

1,600 units **8,000 U/ml** **Lot: 0031412**
RECOMBINANT **Store at –20°C** **Exp: 12/16**

Description: *Bst* 2.0 WarmStart DNA Polymerase is an *in silico* designed homologue of *Bacillus stearothermophilus* DNA Polymerase I, Large Fragment (*Bst* DNA Polymerase, Large Fragment) with a reversibly-bound aptamer, which inhibits polymerase activity at temperatures below 45°C. The aptamer rapidly releases the *Bst* 2.0 WarmStart DNA Polymerase above 45°C and therefore no special activation step is needed to activate the polymerase. *Bst* 2.0 WarmStart DNA Polymerase contains 5´→3´ DNA polymerase activity and strong strand-displacement activity but lacks 5´→3´ exonuclease activity. *Bst* 2.0 WarmStart DNA Polymerase displays improved amplification speed, yield, salt tolerance, and thermostability compared to wild-type *Bst* DNA Polymerase, Large Fragment.

Source: *Bst* 2.0 WarmStart DNA Polymerase is prepared from an *E. coli* strain that expresses the *Bst* 2.0 DNA Polymerase protein from an inducible promoter.

Applications:
- Isothermal amplification (LAMP)
- Applications requiring strand-displacement DNA synthesis
- DNA sequencing through high GC regions
- Rapid sequencing from nanogram amounts of DNA template

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM dithiothreitol, 0.1% Triton® X-100 and 50% glycerol.

Reagents Supplied with Enzyme:
Isothermal Amplification Buffer (10X)
100 mM $MgSO_4$

1X Isothermal Amplification Buffer:
20 mM Tris-HCl
10 mM $(NH_4)_2SO_4$
50 mM KCl
2 mM $MgSO_4$
0.1% Tween® 20
pH 8.8 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 25 nmol of dNTP into acid insoluble material in 30 minutes at 65°C.

Unit Assay Conditions: 50 mM KCl, 20 mM Tris-HCl (pH 8.8), 10 mM $MgCl_2$, 30 nM M13mp18 SS DNA, 70 nM M13 sequencing primer (–47) 24 mer, 200 µM dATP, 200 µM dCTP, 200 µM dGTP, 100 µM dTTP including [^{3}H]-dTTP and 100 µg/ml BSA.

Heat Inactivation: 80°C for 20 minutes.

Protocol:

Typical LAMP Protocol: Incubate the following reaction at 65°C for 30–60 minutes.

COMPONENT	25 µl REACTION	FINAL CONC.
10X Isothermal Amplification Buffer	2.5 µl	1X (contains 2 mM $MgSO_4$)
$MgSO_4$ (100 mM)	1.5 µl	6 mM (8 mM total)
dNTP Mix (10 mM)	3.5 µl	1.4 mM each
FIP/BIP Primers (25X)	1 µl	1.6 µM
F3/B3 Primers (25X)	1 µl	0.2 µM
LoopF/B Primers (25X)	1 µl	0.4 µM
Bst 2.0 WarmStart DNA Polymerase (8,000 U/ml)	1 µl	320 U/ml
DNA Sample	variable	> 10 copies or more
Nuclease-free Water	to 25 µl	
Total Reaction Volume		25 µl

General Guidelines:

1. A LAMP Primer Mix can be prepared with all 4 or 6 (with Loop) primers. A 25X Primer Mix should contain: 40 µM FIP, 40 µM BIP, 5 µM F3, 5 µM B3, 10 µM LoopF, 10 µM LoopB in TE or water.

(see other side)

CERTIFICATE OF ANALYSIS

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(see other side)

CERTIFICATE OF ANALYSIS

2. If analyzing via agarose gel electrophoresis or other method requiring opening LAMP reaction vessels, setup secondary analysis area and equipment to avoid contamination.
3. Running a no-template control is strongly recommended to ensure amplification specificity.
4. If optimization is desired, try titrating Mg^{2+} (4–10 mM final) or *Bst* 2.0 WarmStart DNA Polymerase (0.04–0.32 U/µl), or changing reaction temperature (50–68°C).

Quality Control Assays

Exonuclease Assay: Incubation of a 50 µl reaction in 1X ThermoPol® Reaction Buffer containing a minimum of 500 units of *Bst* 2.0 DNA Polymerase with 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA (10^5 cpm/µg) for 4 hours at 65°C releases < 0.1% of the total radioactivity.

Endonuclease Assay: Incubation of a 50 µl reaction in 1X ThermoPol Reaction Buffer containing a minimum of 500 units of *Bst* 2.0 DNA Polymerase with 1 µg of supercoiled ϕX174 DNA for 4 hours at 65°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

Physical Purity: Purified to > 99% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

Phosphatase Assay: Incubation of a 200 µl reaction in 1 M Diethanolamine (pH 9.8) and 0.5 mM $MgCl_2$ containing 2.5 mM *p*-Nitrophenol Phosphate and a minimum of 100 units of *Bst* 2.0 DNA Polymerase incubated for 4 hours at 37°C yields no detectable phosphatase activity as determined by spectrophotometric analysis of released *p*-nitrophenylene anion at 405 nm.

RNase Activity: Incubation of a 10 µl reaction in 1X NEBuffer 4 containing a minimum of 1 µl of *Bst* 2.0 WarmStart DNA Polymerase and 40 ng of F-300 RNA transcript incubated for 16 hours at 37°C results in < 10% substrate degradation as determined by gel electrophoresis using fluorescent detection.

Enzyme Properties: Activity in NEBuffers

ThermoPol Buffer	125%
Unit Assay Conditions	100%
NEBuffer 1	25%
NEBuffer 2	100%
NEBuffer 3	100%
NEBuffer 4	100%
NEBuffer EcoRI	100%

Notes on Use: *Bst* 2.0 WarmStart DNA Polymerase does not exhibit 3´→ 5´ exonuclease activity.

Reaction temperatures above 70°C are not recommended.

Bst 2.0 WarmStart DNA Polymerase cannot be used for thermal cycle sequencing or PCR.

Companion Products Sold Separately:

Bst 2.0 DNA Polymerase

#M0537S	1,600 units
#M0537L	8,000 units
#M0537M	8,000 units

Bst DNA Polymerase, Large Fragment

#M0275S	1,600 units
#M0275L	8,000 units
#M0275M	8,000 units

Magnesium Sulfate ($MgSO_4$) Solution

#B1003S	6.0 ml

Isothermal Amplification Buffer Pack

#B0537S	6.0 ml

Deoxynucleotide Solution Set

#N0446S	25 µmol each

Deoxynucleotide Solution Mix

#N0447S	8 µmol each
#N0447L	40 µmol each

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4. If optimization is desired, try titrating Mg^{2+} (4–10 mM final) or *Bst* 2.0 WarmStart DNA Polymerase (0.04–0.32 U/µl), or changing reaction temperature (50–68°C).

Quality Control Assays

Exonuclease Assay: Incubation of a 50 µl reaction in 1X ThermoPol® Reaction Buffer containing a minimum of 500 units of *Bst* 2.0 DNA Polymerase with 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA (10^5 cpm/µg) for 4 hours at 65°C releases < 0.1% of the total radioactivity.

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Bst 2.0 DNA Polymerase

#M0537S	1,600 units
#M0537L	8,000 units
#M0537M	8,000 units

Bst DNA Polymerase, Large Fragment

#M0275S	1,600 units
#M0275L	8,000 units
#M0275M	8,000 units

Magnesium Sulfate ($MgSO_4$) Solution

#B1003S	6.0 ml

Isothermal Amplification Buffer Pack

#B0537S	6.0 ml

Deoxynucleotide Solution Set

#N0446S	25 µmol each

Deoxynucleotide Solution Mix

#N0447S	8 µmol each
#N0447L	40 µmol each

ISO 9001 Registered Quality Management
ISO 14001 Registered Environmental Management
ISO 13485 Registered Medical Devices

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Notice to Purchaser: Nucleic acid-based aptamers for use with thermophilic DNA polymerases are licensed exclusively by New England Biolabs, Inc. from SomaLogic, Inc. (See Patent Nos. 5,475,096; 5,670,637; 5,696,249; 5,874,557; and 5,693,502). New England Biolabs, Inc. gives the Buyer/User a non-exclusive license to use the aptamer-based *Bst* 2.0 WarmStart DNA Polymerase for RESEARCH PURPOSES ONLY. Commercial use of the aptamer-based *Bst* 2.0 WarmStart DNA Polymerase requires a license from New England Biolabs, Inc. Please contact busdev@neb.com for more information.

The purchase of NEB *Bst* products conveys to the purchaser the limited, nontransferable right to use the purchased products to perform loop-mediated isothermal amplification ("LAMP") for research use only. LAMP is a patented technology belonging to Eiken Chemical Co., Ltd. and any use other than research may require a license from Eiken Chemical Co., Ltd. A patent is pending for NEB's *Bst* DNA Polymerase.