Product Information	
Bst X DNA Polymerase	
Part Number	P7390L
Concentration	40,000 U/mL
Unit Size	40,000 U
Storage Temperature	-25ºC to -15ºC
Lot Number	(Shipment Specific)
Reference Number	(Shipment Specific)

Product Description: Bst X DNA Polymerase is a thermostable DNA polymerase from Geobacillus, homologous to the DNA polymerase from Bacillus stearothermophilus (Bst). Bst X lacks 5'→3' and 3'→5' exonuclease activity and exhibits a stronger strand-displacement activity than Manta 1.0 DNA Polymerase that may allow it to perform better in isothermal amplification technologies.

Buffer Description: 10x Bst X Xcelerator Reaction Buffer, contains 2 mM MgSO4 at 1X concentration. Depending on the application, it may be necessary to adjust the MgSO4 from 2-10 mM final concentration.

Product Specifications						
P7390						
Assay	**SDS Purity**	**Specific Activity**	**SS Exonuclease**	**DS Exonuclease**	**DS Endonuclease**	**E. coli DNA Contamination**
Units Tested	**N/A**	**N/A**	**4000 U**	**4000 U**	**4000 U**	**4000 U**
Specification	>99%	400,000 U/mg	<5.0% released	<1.0% released	No conversion	< 10 copies

Source of Protein: A recombinant *E.coli* strain carrying the Bst X DNA Polymerase (exo-) polymerase gene.
Unit Definition: 1 unit is defined as the amount of polymerase required to convert 10 nmol of dNTPs into acid insoluble material in 30 minutes at 65°C
Molecular weight: 66,494 Daltons

Quality Control Analysis:
Unit Activity is measured using a 2-fold serial dilution method. Dilutions of enzyme batch were made in 1X reaction buffer and added to 50 µL reactions containing Calf Thymus DNA, 1X PCR Buffer II, 3H-dTTP and 100 µM dNTPs. Reactions were incubated 10 minutes at 65°C, plunged on ice, and analyzed using the method of Sambrook and Russell (Molecular Cloning, v3, 2001, pp. A8.25-A8.26).
Protein Concentration is determined by OD_{280} absorbance.
Physical Purity is evaluated by SDS-PAGE of concentrated and diluted enzyme solutions followed by silver stain detection. Purity is assessed by comparing the aggregate mass of contaminant bands in the concentrated sample to the mass of the protein of interest band in the diluted sample.
Single-Stranded Exonuclease is determined in a 50 µL reaction containing a radiolabeled single-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.
Double-Stranded Exonuclease is determined in a 50 µl reaction containing a radiolabeled double-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.
Double-Stranded Endonuclease is determined in a 50 µL reaction containing 0.5 µg of plasmid DNA and 10 µL of enzyme solution incubated for 4 hours at 37°C.
E.coli 16S rDNA Contamination is evaluated using 5 µL replicate samples of enzyme solution denatured and screened in a TaqMan qPCR assay for the presence of contaminating *E.coli* genomic DNA using oligonucleotide primers corresponding to the 16S rRNA locus.
Supplied in: 50 mM KCl, 1mM DTT, 0.1mM EDTA, <0.1% Triton X-100, 50% glycerol, 10mM Tris-HCl pH 7.5 @ 25ºC.
Supplied with: 10x Bst X Xcelerator Reaction Buffer (B7390L) and 100 mM $MgSO_4$ solution (B0320L)

Limitations of Use
This product was developed, manufactured, and sold for *in vitro* use only. The product is not suitable for administration to humans or animals. MSDS sheets relevant to this product are available upon request.

Notes:

Alternate Temperature

- Approximately 15% activity at 37°C.
- Approximately 5% activity at 25°C.

Usage Notes:

- Optimal reaction temperature from 60-70°C.
- Heat inactivated at 80°C after 10 min incubation.
- High Tolerance to the non-ionic detergents Tween-20, Tween-80, TX-100, and NP-40 (up to 5% final concentration).
- Active in salt from 40 to 125 mM.

LAMP Protocol using Bst X DNA Polymerase:

Loop-mediated isothermal amplification (LAMP) is a common method for accurate and cost-effective detection of a variety of pathogens, particularly in point-of-care environments. The method requires a strand-displacing DNA polymerase and a set of four to six specialized primers that recognize several distinct regions on the target DNA. Amplification occurs at a constant temperature and is a highly sensitive and rapid process, creating long and repeated concatemers that can be analyzed by real-time fluorescence, turbidimetry, colorimetric assays, or by lateral flow methods.

Materials needed

- Bst X DNA polymerase (40,000 U/ml), P7390L
- 10X Xcelerator Reaction Buffer, B7390L
- 100 mM $MgSO_4$ solution, B0320L
- 10 mM dNTP solution mix, 201900
- 10X LAMP Primer Mix, user supplied
- 20X EvaGreen Dye, Biotium #31000 or other fluorescent DNA-binding dye (optional, for real-time detection)
- Real-time fluorimeter or PCR instrument (optional, for real-time detection)

Guidelines for Reation Setup

Use general precautions when assembling reactions to avoid cross-contamination, particularly if the analysis method requires that completed reaction tubes be unsealed.

To prepare a 10X LAMP primer mix, the six specialized primers can be combined at the following concentrations: 10 µM FIP, 10 µM BIP, 1 µM F3, 1 µM B3, 5 µM LoopF, 5 µM LoopB in water or TE buffer. It may be useful to adjust primer concentrations for optimal reaction performance.

Assemble reactions on ice in the following order:

Component	Volume	Final concentration
Nuclease-free water	Variable	-
10X Xcelerator Reaction Buffer	2.5 ul	1X (provides 2 mM MgSO4)
10X LAMP Primer Mix	2.5 µl	1X (1 µM FIP/BIP, 0.1 µM F3/B3, 0.5 µM LoopF/LoopB)
10 mM dNTP solution mix	3.5 µl	1.4 mM each
100 mM MgSO4 solution	Up to 2 µl	Up to 8 mM additional (10 mM final)
20X EvaGreen dye (optional)	0.3 µl	0.25X
Sample template	Up to 5 µl	Variable
Bst X DNA polymerase	1 µl	1.6 U/µl
Final volume	**25 µl**	

If a different final reaction volume is required, please scale all components proportionally.

Vortex gently to mix contents, then briefly centrifuge to collect components at the bottom of the reaction tube.

Incubate reactions at 65°C in a water bath, heat block, thermocycler, or real-time fluorimeter or qPCR instrument for real-time fluorescence measurements. Reactions are typically complete within 5 to 40 minutes.

Optimization Guidelines

$MgSO_4$: The 10X Reaction buffer provides 2 mM $MgSO_4$ to reactions, but additional magnesium may be required for optimal speed and reaction efficiency (2 mM to 8 mM). Excess magnesium can negatively impact primer specificity.

Bst X DNA polymerase: Recommended starting concentration is 1 µl per 25 µl reaction (1.6 U/ul). The concentration can be adjusted from 0.4 U/µl to 1.6 U/µl if optimization is required.

Reaction temperature: Standard reactions should be run at 65°C for optimal performance, but optimization can be conducted at a range of 60°C to 70°C.

Legal Disclaimer: Patents

Certain applications in which this product can be used may be covered by patents issued and applicable in the United States and abroad. Purchase of this product does not include a license to perform any patented application; therefore it is the sole responsibility of users of this product to determine whether they may be required to engage a license agreement depending upon the particular application in which the product is used.

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