Manual

Bst DNA Polymerase, Exonuclease Minus

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Bst DNA Polymerase, Exonuclease Minus is part of the Epicentre™ product line, known for its unique genomics kits, enzymes, and reagents which offer high quality and reliable performance.

BIOSEARCH™ TECHNOLOGIES

GENOMIC ANALYSIS BY LGC

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1. Introduction

Bst DNA Polymerase, Exonuclease Minus, is a recombinant form of the 67 kDa *Bacillus stearothermophilus* DNA Polymerase protein (large fragment). The enzyme has 5′→3′ polymerase activity and strand displacement activity, but it lacks 3′→5′ exonuclease activity. It also has reverse transcription activity.

Bst DNA Polymerase, Exonuclease Minus from LGC, Biosearch Technolgies™, has higher strand displacement activity than that of other suppliers (Figure 1). The enzyme can be used in nucleic acid amplification methods (see Notice to Purchasers) such as isothermal amplification, whole genome amplification (WGA), and multiple displacement amplification (MDA). It also can be used in next generation sequencing.

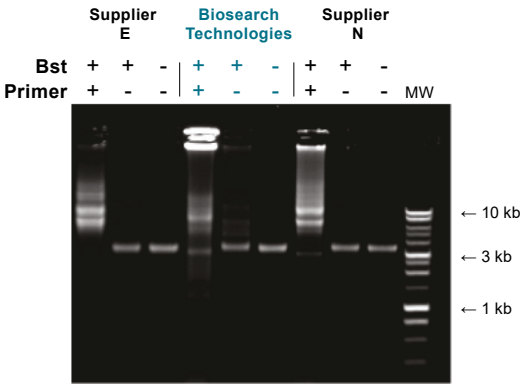

Figure 1. Biosearch Technologies' Bst DNA Polymerase, Exonuclease Minus, possesses greater strand-displacing polymerase activity. M13 single stranded DNA was incubated with or without 8 units of Bst DNA Polymerase (+/- Bst) in reaction buffer supplied by the manufacturer, with or without replication primer (+/- primer) for 30 minutes at 65 °C. MW, 1 kb ladder.

This enzyme has optimal activity at 65 °C. It is suitable for sequencing DNA with high GC content and secondary structures. It is available in concentrations of 8,000 U/mL or 50,000 U/mL.

2. Product designations and kit components

Product	Kit size	Catalogue number	Part number	Reagent description	Part number	Volume
Bst DNA Polymerase, Exonuclease Minus	200 Units	30027-0	A93635-0	Bst DNA Polymerase, Exonuclease Minus (8,000 U/mL)	F93635-0	25 µL
				10X DNA Polymerase Buffer B	F98637-1	1.2 mL
	2,000 Units	30027-1	A93635-1	Bst DNA Polymerase, Exonuclease Minus (8,000 U/mL)	F93635-1	250 µL
				10X DNA Polymerase Buffer B	F98637-1	1.2 mL
	10,000 Units	30027-2	A93635-2	Bst DNA Polymerase, Exonuclease Minus (8,000 U/mL)	(5X) F93635-1	1.25 mL
				10X DNA Polymerase Buffer B	(5X) F98637-1	6 mL

3. Product specifications

Storage: Store only at -20 °C in a freezer without a defrost cycle.

Storage buffer: 10 mM Tris-HCl, pH 7.5, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.1% Triton® X-100 (Rohm & Haas) and 50% Glycerol.

Stability: Bst DNA Polymerase, Exonuclease Minus is stable for one year from the date received if stored at -20 °C.

Recommended reaction conditions: 8 U Bst DNA Polymerase, Exonuclease Minus; 1X DNA Polymerase Buffer B containing 20 mM Tris-HCl pH 8.8, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$ and 0.1 % Triton® X-100.

Activity determination: One unit catalyses the incorporation of 10 nmol of dNTP into acid-insoluble material in 30 minutes at 65 °C in 20 mM Tris-HCl pH 8.8 , 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, 0.1 % Triton X-100, 30 nM M13mp18 ssDNA, 70 nM M13 sequencing primer(-47) 24 mer, 200 µM dGTP, dATP, dTTP, dCTP (a mix of unlabeled and [^{33}P]dCTP) and 0.1 mg/mL BSA.

Absence of endonuclease or nicking activity: Incubation of 8 U of Bst DNA Polymerase, Exonuclease Minus with 1 µg of pUC19 DNA for 16-18 hours at 37 °C resulted in no smearing of bands as detected by agarose gel electrophoresis.

Absence of exonuclease activity: Incubation of 8 U of Bst DNA Polymerase, Exonuclease Minus with 1 µg of HindIII-cut lambda DNA for 16 hours at 37 °C and 65 °C resulted in no smearing of bands on agarose gels. Single stranded and double stranded exonuclease activities were tested by incubating 10 µL of enzyme at 8 U/ µL with radiolabeled DNA substrate for one hour at 37 °C and 65 °C, resulting in less than 0.1% release of TCA-soluble counts.

Purity: >90% pure by SDS PAGE. No detectable DNA contamination. 10 µL of enzyme at 8 U/ µL of the sample was tested for *E. coli* genomic DNA contamination by PCR amplifying with the *E. coli* 16S ribosomal primers.

Heat inactivation: 80 °C for 20 min.

4. Applications

- Strand displacement amplification (see Notice to Purchasers)
- DNA sequencing through high GC regions[1,2]
- Rapid sequencing from nanogram amounts of DNA template[3]

5. DNA LAMP Protocol

5.A. Suggested Setup

2.5	µL 10X DNA Polymerase buffer B*	(1X final concentration)
1.5	µL 100 mM $MgSO_4$	(6 mM final concentration; total 8 mM)
3.5	µL 10 mM dNTPs mix	(1.4 mM final concentration each)
1.25	µL Target specific primers, 20X	(1X final concentration)
x	µL F3 and B3 Primers	(200 nM final concentration each)
y	µL Loop F and B Primers	(800 nM final concentration each)
1.0	µL Bst DNA Polymerase, Exonuclease Minus, 8U/µL	(8 U/rxn)
z	µL DNA Template	(10 copies or more)
25	µL Total reaction volume	

*1X Buffer B contains 2 mM $MgSO_4$

5.B. Workflow

Note: *In order to minimise cross-contamination, steps 6 onward should be done in an area separate from the area where you are preparing the reaction mix.*

1. Thaw all kit components and hold on ice.
2. **All components should be mixed well before use. Vortex all tubes for 10 seconds, then centrifuge briefly to collect.**
3. Prepare the reaction mix as shown in the Suggested Setup in the order listed. Add all the components except the DNA template. *During this step the reaction mix tube should always be held on the ice to prevent the background activity of the enzyme.*
4. After all reagents have been added, mix the reaction completely. Gently vortex. *This step is required to ensure uniform distribution of all reaction components.*

5. Dispense reaction mix (25 µL minus sample volume) into a PCR tube or 96-well PCR plate well for each reaction.
6. Add volume of DNA template to each well/tube for a total volume of 25 µL per well.
7. Run a no-template control (negative control) to ensure amplification specificity.
8. Cap tubes or seal plate wells. Centrifuge briefly to collect prior to incubation.
9. Incubate reactions at desired temperature for 30-40 minutes. *Running a temperature gradient from (55-65 °C) is strongly recommended to determine optimum temperature.*
10. If required, run samples on a 2% agarose gel.
11. If optimisation is desired, try titrating Mg^{2+} (4-10 mM final) or Bst DNA Polymerase, Exonuclease Minus (0.04-0.32 U/µL).

Note: *Reactions may be kept at -20 °C for longer term storage.*

5.C. Tips for use:

- Requires 0.1% Triton X-100 for long term storage.
- Reaction temperatures above 70 °C are not recommended.
- Bst DNA Polymerase cannot be used for thermal cycle sequencing.

6. References

1. Griffin, H. and Griffin, A. (1994) *PCR Technology*, 228-229.
2. McClary, J. et al. (1991) *J. DNA Sequencing and Mapping,* **1,** 173-180.
3. Mead, D.A. et al. (1991) *Biotechniques*, **11,** 76-87.

7. Further support

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com

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