Bst DNA Polymerase, Exonuclease Minus

8,000 U/mL

Cat. No. P1111 200 Units Trial Size (1 x 25 µL)

Cat. No. P1112 2,000 Units (1 x 250 µL)

Cat. No. P1113 10,000 Units (5 x 250 µL)

Includes 10X DNA Polymerase Buffer B (1.2 ml per 2,000 Units)

Store at –20 ºC.

For Research Use Only. Not for use in Diagnostic Procedures.

Technical Specifications

Product Description

Bst DNA Polymerase, Exonuclease Minus, 8,000 units/mL.

Storage Buffer

10 mM Tris-HCl, pH 7.5, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.1% Triton X-100, and 50% Glycerol.

Stability

Bst DNA Polymerase, Exonuclease Minus is stable for one year from the date received if stored at –20 ºC.

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 $(NH4)^2SO^4$, 10 mM KCl, 2 mM $MgSO^4$,and 0.1 % Triton X-100.

Activity Determination

One unit catalyzes 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 $(NH4)_2SO_4$,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 ºC 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 ºC and 65 ºC 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/ µLwith radiolabeled DNA substrate for one hour at 37 ºC and 65 ºC, 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.

DNA LAMP Protocol

Suggested set up:

Component	Final Conc.	Volume, µL
10X DNA Polymerase buffer B*	1X	2.5
100 mM MgSO4	6 mM (total 8 mM)	1.5
10 mM dNTPs mix	1.4 mM each	3.5
Target specific primers, 20X	1X	1.25
F3 and B3 Primers	200 nM each	
Loop F and B Primers	800 nM each	
FIP and BIP Primers	1600 nM each	
Bst DNA Polymerase, Large fragment, 8U/µL	8 U/rxn	1.0
DNA sample	10 copies or more	Variable
Nuclease free Water		To 25 µL

*1X Buffer B contains 2 mM $MgSO_4$

Workflow:

In order to minimize 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 Table 1 in the order listed. Add all the components except the target. 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 target 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 optimization is desired, try titrating Mg^{2+} (4–10 mM final) or *Bst* DNA Polymerase, Large Fragment (0.04–0.32 U/µL).

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

Applications

1. DNA sequencing through high GC regions (1, 2)

2. Rapid Sequencing from nanogram amounts of DNA template (3)

Heat Inactivation: 80 ºC for 20 min.

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.