

Bst DNA Polymerase, Exonuclease Minus 50,000 U/ml Technical Specifications



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Catalog No. 30028-1 10,000 Units (200µl @ 50 U/ µl)
Includes 10X DNA Polymerase Buffer B (4 X 1.2 ml)
Store at -20°C.
For Research Use Only.
Not for use in Diagnostic Procedures.

Product Description	Bst DNA Polymerase, Exonuclease Minus, 50,000 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°C.
Recommended Reaction Conditions	50 U Bst DNA Polymerase, Exonuclease Minus; 1X DNA Polymerase Buffer B containing 20 mM Tris-HCl pH 8.8, 10 mM (NH ₄) ₂ SO 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 (NH ₄) ₂ SO 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 50 U of Bst DNA Polymerase, Exonuclease Minus with 1 µg of supercoiled pBR322 DNA for 16 hours at 37° and 65°C resulted in no detectable conversion to relaxed or linear forms by agarose gel electrophoresis.
Absence of Exonuclease Activity	Incubation of 50 U of Bst DNA Polymerase, Exonuclease Minus with 1 µg of HindIII-cut lambda DNA for 16 hours at 37° 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 50 U/ µl with radiolabeled DNA substrate for one hour at 37° and 65°C, resulting in less than 0.1% release of TCA-soluble counts.
Purity	>99% pure by SDS PAGE. No detectable DNA contamination. 10 µl of enzyme at 50 U/ µl of the sample was tested for <i>E. coli</i> genomic DNA contamination by PCR amplifying with the <i>E. coli</i> 16S ribosomal primers.

Applications

1. DNA sequencing through high GC regions (1, 2)
2. Rapid Sequencing from nanogram amounts of DNA template (3)

Heat Inactivation: 80°C 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.

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