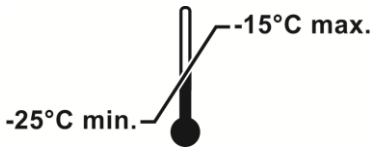
Bst DNA Polymerase, Exonuclease Minus 50,000 U/ml

Technical Specifications

Catalog No. 30028-1 10,000 Units (200µl @ 50 U/ µl)
Includes 10X DNA Polymerase Buffer B (4 X 1.2 ml)



Store at –20ºC.
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 –20ºC.
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 $(NH_4)_2SO_4$, 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 $(NH_4)_2SO_4$, 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 50 U of Bst DNA Polymerase, Exonuclease Minus with 1 µg of supercoiled pBR322 DNA for 16 hours at 37º and 65ºC 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 37º and 65ºC 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 37º and 65ºC, 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 *E. coli* genomic DNA contamination by PCR amplifying with the *E. coli* 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: 80ºC 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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MA074 Rev. B

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