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Certificate of Analysis

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Bst DNA Polymerase

Catalog No: 1078

Lot No: See Product Label
Package Size: See Product Label
Concentration: See Product Label
Protein (Bradford): 0.102 mg/ml
Specific Activity: 97,859 units/mg
Storage Conditions: Store at -20°C
Notes: Avoid multiple freeze / thaw cycles or frequent temperature changes

Description

- Large exonuclease free fragment of thermophilic Bst DNA Polymerase with strand displacement activity
- Moderately thermostable enzyme from Bacillus stearothermophilus.
- Exhibits thermophilic reverse transcriptase activity.
- Active over a wide range of reaction buffer conditions and magnesium ion concentrations.
- Ultrapure recombinant protein.

Applications

- Ideal for DNA synthesis reactions requiring strand displacement.
- Used in isothermal nucleic acids amplification.
- Used in isothermal DNA sequencing at elevated temperatures.
- Catalyzes the polymerization of nucleotides into duplex DNA in the 5'→3' direction in the presence of magnesium ions.
- Lacks the 5'→3' exonuclease activity, while retaining the polymerase activity (1).
- Broad activity range; can replace mesophilic polymerases as well as synthesize DNA at high temperatures, therefore suitable for amplification of difficult DNA templates, including repetitive sequences, GC-rich regions and problematic secondary structures (2,3).
- Can be heat inactivated at temperatures above 80°C.
- Replicates DNA optimally at 65°C.

Unit Definition

One unit is the amount of enzyme required to incorporate 10.0 nmol of total nucleotide into an acid insoluble form in 30 minutes at 60ºC under the stated assay conditions.

Assay Conditions

50 mM Tris-HCl (pH 8.6)
100 mM KCl
10 mM magnesium chloride
1 mM dithiothreitol
0.02 mM of each dCTP, dGTP, dTTP, dATP, and 0.2 mM [α-^{32}P]dATP
50 µg bovine serum albumin
15 µg activated calf thymus DNA
Reaction volume of 50 µl

Storage Buffer

20 mM potassium phosphate (pH 7.1)
1 mM DTT
50% (v/v) glycerol

Quality Control

Nicking: Incubation of 5, 10 and 20 units of Bst DNA Polymerase with 1 µg of pBR322 DNA at 60ºC for 1 hour resulted in ≤5% conversion of RFI to RFII DNA and no conversion RF III. Reaction volume of 50 µl.

Purity: ≥90% pure, as judged by SDS-polyacrylamide gel electrophoresis.

DNase, double-stranded: Incubation of 5, 10,

and 20 units of enzyme with 0.015 µg of [^{32}P]lambda DNA for 1 hour at 60°C resulted in a ≤1.0 slope of %-end label released per unit of enzyme. Reaction volume of 20 µl.

5'-Exonuclease/5'-Phosphatase: Incubation of 5, 10, and 20 units of enzyme with 0.25 µg of 5'-ends of [5'-^{32}P] lambda/Hae III DNA fragments for 1 hour at 60°C resulted in ≤0.5 slope of %-end label released per unit of enzyme. Reaction volume of 50 µl.

DNase, single-stranded: Incubation of 5, 10, and 20 units of enzyme with 0.015 µg of heat denatured [^{32}P]lambda DNA for 1 hour at 60°C resulted in a ≤1.0 slope of %-end label released per unit of enzyme. Reaction volume of 20 µl.

References

(1) Stenesh, J. and Roe, B.A. (1972) Biochim. Biophys. Acta. 272, 156-166
(2) Hugh, G. and Griffin, M. (1994) PCR Technology, p.p. 228-229
(3) McClary, J. et al. (1991) J. DNA Sequencing and Mapping, p.p. 173-180