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Certificate of Analysis

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Thermus (Tfl) DNA Polymerase

Catalog No: 1112

Lot No: See Product Label

Package Size: See Product Label

Concentration: See Product Label

Storage Conditions: Store at -20°C

Applications

- Efficiently synthesizes DNA at elevated temperatures (1)
- Used for high temperature DNA sequencing
- Broader Mg²⁺ ions concentration tolerance than taq DNA polymerase
- Can be used for amplification of nucleic acids in PCR and LCR reaction

Unit Definition

One unit is the amount of enzyme required to incorporate 10 nmoles total nucleotide into acid insoluble material at 70°C for 30 minutes

Assay Conditions

25 mM Tris-HCl (pH 9.5 at 25°C)
50 mM potassium chloride
10 mM MgCl₂
1 mg/ml bovine serum albumin
300 µg/ml activated calf thymus DNA
0.2 mM each dCTP, dGTP, dTTP and [α -³²P]
dATP
Incubation is at 70°C for 10 minutes in a
reaction volume of 50 µl.

Storage Buffer

50 mM Tris-HCl (pH 7.5)
0.1 mM EDTA
5 mM dithiothreitol,
50% (v/v) glycerol
Stabilizers

Quality Control

DNase, double-stranded: Incubation of 2.5, 5, and 10 units of Thermus DNA Polymerase with 0.03 µg of [³²P]lambda DNA at 70°C for 1 hour resulted in ≤0.6 slope of %-end label released per unit of enzyme. Reaction volume of 50 µl.

DNase, single-stranded: Incubation of 2.5, 5, and 10 units of Thermus DNA Polymerase with 0.03 µg of heat denatured [³²P]lambda DNA at 70°C for 1 hour resulted in ≤0.6 slope of %-end label released per unit of enzyme. Reaction volume of 50 µl.

3'-Exonuclease: 5, 10, and 20 units of Thermus DNA Polymerase and 5 pmoles of 3'-ends of lambda/Taq I fragments (3'-labelled with Klenow exo- and [³H]dCTP), incubated for 1 hour at 70°C resulted in ≤0.6 slope of %-end label released per unit of enzyme. Reaction volume of 50 µl.

Nicking: Incubation of 5, 10, and 20 units of Thermus DNA Polymerase with 1.0 µg of pBR322 DNA at 70°C for 1 hour resulted in ≤10% conversion of RFI to RFII DNA. Reaction volume of 50 µl.

References

(1) Kaledin, A. S., Sliusarenko, A. G. and Gorodetskii, S. I. (1980) *Biokhimiya* 45, 644-651