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Certificate of Analysis

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Tth DNA Polymerase

Thermus thermophilus-111

Catalog No: 1115

Lot No: See Product Label
Package Size: See Product Label
Concentration: See Product Label
Storage Conditions: Store at -20°C

Applications

- Can reverse transcribe at elevated temperatures
- Minimizes problems with strong secondary structure of RNA
- Results in greater specificity of primer hybridization and extension
- Suitable for high temperature synthesis of DNA
- Synthesizes cDNA from RNA template
- Stable thermophilic DNA polymerase (1)

Unit Definition

One unit is the amount of enzyme required to incorporate 10 nmoles total nucleotides into acid-insoluble material in 30 minutes at 70°C.

Assay Conditions

25 mM Tris-HCl (pH 9.5 at 25°C)
50 mM potassium chloride
10 mM magnesium chloride
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
5.0 mM dithiothreitol
50% (v/v) glycerol
Stabilizers

Quality Control

DNase, double-stranded: Incubation of 2.5, 5 and 10 units of Tth DNA Polymerase with 0.003 µg of [³²P] lambda DNA at 70°C for 1 hour resulted in ≤ 0.5-slope of %-label released per unit of enzyme. Reaction volume of 50 µl.

DNase, single-stranded: Incubation of 2.5, 5 and 10 units of Tth DNA Polymerase 0.03 µg of [³²P] lambda DNA at 70°C for 1 hour resulted in ≤ 0.5-slope -slope of %-label released per unit of enzyme. Reaction volume of 50 µl.

3'-Exonuclease: 5, 10 and 20 units of Tth DNA Polymerase and 5 pmoles of 3'-ends of lambda/TaqI fragments (3'-labelled with Klenow exo- and [³H] dCTP), incubated for 1 hour at 70°C resulted in ≤ 0.5-slope of % end label released per unit of enzyme. Reaction volume of 50 µl.

5'-Exonuclease/5'-Phosphatase: Incubation of 5, 10 and 20 units of Tth DNA Polymerase with 0.25 µg of 5'-ends of [5'-³²P] lambda/Hae III fragments for 1 hour at 37°C resulted in ≤ 0.5 slope of %-end label released per unit of enzyme. Reaction volume of 50 µl.

Nicking: Incubation of 5, 10 and 20 units of Tth DNA Polymerase with 0.5 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) Wang, A.M., Doyle, M.V., and Mark, D.F. (1989) *Proc. Nat'l. Acad. Sci. USA* 86, 9717-9721

For more information or to re-order, please visit www.chimerx.com