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Certificate of Analysis

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Tth DNA Reverse Transcriptase

Catalog No: 1374

Lot No: See Product Label

Package Size: See Product Label

Concentration: 3.0 units/ μ l

Source: *Thermus thermophilus*-111

Storage Conditions: Store at -20°C

Storage Buffer

50 mM Tris-HCl (pH 7.5)

0.1 mM EDTA

5.0 mM dithiothreitol

50% (v/v) glycerol

Stabilizers

Applications

- Suitable for high temperature synthesis of DNA (1)
- Synthesizes cDNA from RNA template (2)
- Results in greater specificity of primer hybridization and extension of RNA
- Can reverse transcribe at elevated temperatures (2)
- Minimizes problems with strong secondary structure of RNA
- Used for efficient PCR of DNA, containing problematic secondary structures
- Applicable to RT-PCR; the same enzyme is used for both reverse transcription and following amplification of obtained cDNA template (2)
- Resistant to amplification inhibitors present in template DNA isolated from problematic samples (3,4)

Unit Definition

One unit is the amount of enzyme required to incorporate 1.0 nmole TTP into acid-insoluble material in 10 minutes at 50°C .

Assay Conditions

40 mM Tris-HCl (pH 8.5)

1 mM MnCl_2

10 mM dithiothreitol

50 mg/ml bovine serum albumin

0.4mM polyA(dT)

.5 mM ($\alpha^{32}\text{P}$)TTP at 10 $\mu\text{Ci/ml}$.

Incubation is at 50°C for 10 minutes in a reaction volume of 50 μl .

Quality Control

DNase, double stranded: Incubation of 2.5, 5, and 10 units of enzyme with 0.03 μg of [^{33}P]lambda DNA at 70°C for 1 hour resulted in -0.064 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 enzyme with 0.03 μg of heat denatured [^{33}P]lambda DNA at 70°C for 1 hour resulted in -0.087 slope of %-end label released per unit of enzyme. Reaction volume of 50 μl

RNase: Incubation of 2.5, 5, and 10 units of enzyme with 0.015 μg of [^{32}P] RNA transcript for 1 hour at 70°C resulted in ≤ 0.4 slope of %-end label released per unit of enzyme. Reaction volume of 50 μl

3'-Exonuclease: Incubation of 5, 10, and 20 units of enzyme and 0.13 μg of 3'-ends of lambda/Taq I fragments (3'-labeled with T4 DNA Polymerase and [^3H]dGTP and [^3H]dCTP), incubated for 1 hour at 70°C resulted in 0.116 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 enzyme with 0.05 μg of 5'-ends of [^{33}P]lambda/Hae III fragments for 1 hour at 70°C resulted in -0.011 slope of %-end label released per unit of enzyme. Reaction volume of 50 μl

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Nicking: Incubation of 5, 10, and 20 units of enzyme 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

Purity: >95% pure as judged SDS-polyacrylamide gel electrophoresis.

References

- (1) Wang, A.M., Doyle, M.V. and Mark, D.F. (1989) *Proc. Natl. Acad. Sci. USA* 86, 9717-9721
- (2) Myers, T.W., Gelfand, D.H. (1991) *Biochem.* 30, 7661-7666
- (3) Kather, H.L. and Schwartz, I. (1994) *Biotechniques* 16, 84-92
- (4) Poddar, S.K., Sawyer, M.H. and Connor, J.D. (1998) *J. Med. Microbiol.* 47, 1131-1135

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