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Certificate of Analysis

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AMV Reverse Transcriptase (Native)

Catalog No: 1372

Lot No: See Product Label

Package Size: See Product Label

Concentration: See Product Label

Storage Conditions: Store at -20°C

Description

- RNA dependent polymerase that synthesizes a complementary DNA strand from a single-stranded RNA or DNA template in the presence of a primer
- Contains both reverse transcriptase and RNase H activities
- Maintains RNase H activity necessary for cDNA synthesis and isothermal RNA amplification

Applications

- Can be used for preparing labeled hybridization probes
- Ideal for use in RT-PCR, RAMP™, NASBA™, cDNA libraries and dideoxy-DNA sequencing

Unit Definition

One unit is the amount of enzyme required to incorporate 1 nmol of labeled dTTP into acid-insoluble material in 10 minutes at 37°C.

Assay Conditions

50 mM Tris-HCl (pH 8.3)
10 mM MgCl₂
40 mM KCl
0.5 mM [³H] dTTP
0.4 mM poly(rA)•(dT)₅₀
Reaction volume of 100 µl

Storage Buffer

0.2 M potassium phosphate (pH 7.2)
2 mM dithiothreitol
0.2% Triton X-100™
50% (v/v) glycerol

Quality Control

Nicking: Incubation of 20, 40, and 80 units of enzyme with 1.0 µg of pBR322 DNA at 37°C for 1 hour resulted in ≤10% conversion of RFI to RFII DNA. Reaction volume of 50 µl.

5'-Exonuclease/5'-Phosphatase: Incubation of 20, 40, and 80 units of enzyme with 0.25 µg of 5'-ends of [5'-³²P]lambda/Hae III DNA fragments for 1 hr at 37°C resulted in a ≤0.4 slope of %-end label released per unit of enzyme. Reaction volume of 50 µl.

3'-Exonuclease: Incubation of 20, 40, and 80 units of enzyme with 5 pmoles of 3'-ends of lambda/Taq I fragments (3'-labeled with Klenow exo- and [³H]dCTP), incubated for 1 hour at 37°C resulted in a ≤0.4 slope of %-end label released per unit of enzyme. Reaction of volume 50 µl.

DNase, double-stranded: Incubation of 20, 40, and 80 units of enzyme with 0.03 µg of [³²P]lambda DNA at 37°C for 1 hour resulted in ≤0.4 slope of %-end label released per unit of enzyme. Reaction volume of 20 µl.

DNase, single-stranded: Incubation of 20, 40, and 80 units of enzyme with 0.03 µg of heat denatured [³²P]lambda DNA at 37°C for 1 hour resulted in ≤0.4 slope of %-end label released per unit of enzyme. Reaction volume of 20 µl.

RNase: Incubation of 20, 40, and 80 units of enzyme with 0.015 µg of [³²P] RNA transcript for 1 hour at 37°C resulted in ≤0.4 slope of %-end label released per unit of enzyme. Reaction volume of 50 µl.

Purity: > 90% pure, as judged by silver stain SDS-polyacrylamide gel electrophoresis.

References

- (1) Goodman, H.M. and MacDonald, R.J. (1979) *Methods Enzymol.* 69, 75-90.
- (2) Naylor, L.H. and van de Sande, J.H. (1986) *Nucleic Acids Res.* 14, 5939
- (3) Zagursky, R.J., Baumeister, K., Lomax, N. and Berman, M.L. (1985) *Gene Anal. Techn.* 2, 89-94
- (4) Houts, G.E., Masakau, M., Ellis, C., Beard, D. and Beard, J.W. (1979) *J. Virol.* 29, 517-522

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