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Certificate of Analysis

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Human DNA Polymerase Alpha

Catalog No: 1075

Lot No: 2404003

Package Size: See Product Label

Concentration: 5 units/ μ l

Protein (Bradford): 0.297 mg/ml

Primase Activity: Present

Specific Activity: 16,835 units/mg

Storage Conditions: Store at -80°C

Note: Avoid multiple freeze / thaw cycles
or frequent temperature changes

Unit Definition

One unit is the amount of enzyme required to incorporate 1 nmol of total nucleotide into acid-insoluble form in 60 minutes at 37°C .

Storage Buffer

20 mM Tris-HCl (pH 8.0)

0.25 mM EDTA

50 mM NaCl

1.0 mM β -mercaptoethanol

0.1% Triton X-100™

50% (v/v) glycerol

Reaction Conditions

60 mM Tris-HCl (pH 8.0)

5 mM magnesium acetate

0.3 mg/ml bovine serum albumin

1 mM dithiothreitol

0.1 mM spermine

0.05 mM of each dCTP, dGTP, dTTP,

and $[\alpha\text{-}^{32}\text{P}]\text{dATP}$

10 μ g activated DNA

Reaction volume is 50 μ l

Quality Control

Protein Concentration: Protein concentration was determined using the Bradford Assay.

Protein Purity: 150 ng were evaluated by SDS-PAGE/silver staining for the presence of four bands at 48, 58, 70, and 180 kDa.

Nicking: 1, 2.5, and 5 units of DNA polymerase α were incubated with pBR322 DNA for 1 hour at 37°C .

DNase: 1, 2.5, and 5 units of DNA polymerase α were incubated with $[\text{}^{32}\text{P}]$ labeled DNA substrate (both single- and double-stranded) for 1 hour at 37°C .

RNase: 1, 2.5, and 5 units of DNA Polymerase Alpha were incubated with $[\text{}^{32}\text{P}]$ labeled RNA transcript for 1 hour at 37°C .

5'-Exonuclease/5'-Phosphatase: 1, 2.5, and 5 units of DNA Polymerase Alpha were incubated with $[\gamma\text{-}^{32}\text{P}]$ labeled 5'-ends of Lambda/Hae III fragments for 1 hour at 37°C .

3'-Exonuclease: 1, 2.5, and 5 units of DNA Polymerase Alpha were incubated with $[\text{}^3\text{H}]$ labeled 3'-ends of Lambda/Taq I DNA fragments for 1 hour at 37°C .