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# Certificate of Analysis

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# Terminal Deoxynucleotidyl Transferase

**Catalog No:** 1390

**Lot No:** See Product Label
**Package Size:** See Product Label
**Concentration:** See Product Label
**Protein (Bradford):** 0.164 mg/ml
**Specific Activity:** 30,568 units/mg
**Storage Conditions:** Store at -20°C
**Note:** All reactions should be run in polypropylene tube.

## Applications

- Prefered substrates are single-stranded DNA, double-stranded DNA with 3'-hydroxyl termini and oligodeoxynucleotide primers (1)
- Used for specific labeling of 3'-termini with ribonucleotides (2)
- Labels 3'-ends of DNA fragments with an [α-32P] 3'-deoxynucleoside (3)
- Adds homopolymer tails of deoxyribonucleotides to vectors or cDNAs (4,5)

## Unit Definition

One unit is the amount of enzyme required to transfer 1 nmol of dAMP from dATP to the 3'-OH terminus of the oligonucleotide initiator $d(A)_{15}$ in 1 hour at 37°C.

## Assay Conditions

40 mM K-Cacodylate (pH 7.2)
8 mM $MgCl_2$
0.33 mM $ZnSO_4$
10 µM oligonucleotide $d(A)_{50}$
BSA buffer (10µl)
1 mM ($\alpha$ $^{32}P$) dATP
Reaction volume of 60 µl

## Storage Buffer

50 mM Potassium phosphate (pH 7.4)
1 mM β-mercaptoethanol
50% (w/v) glycerol

## Quality Control

**Nicking:** Incubation of 10, 20, and 40 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.

**3'-Exonuclease:** Incubation of 10, 20, and 40 units of enzyme with 5 pmoles of 3'-ends of lambda/Taq I fragments (3'-labeled with Klenow [$^{3}H$]dCTP), incubated for 1 hour at 37°C resulted in a ≤1.0 slope of %-end label released per unit of enzyme. Reaction volume of 50 µl.

**5'-Exonuclease/5' Phosphatase:** Incubation of 10, 20, and 40 units of enzyme with 0.25 pmoles of 5'-ends of [5' $^{32}P$] lambda/HaeIII DNA fragments for 1 hour at 37°C resulted in a ≤1.0 slope of %-end label released per unit enzyme. Reaction volume of 50 µl.

**DNase, double-stranded:** Incubation of 10, 20, and 40 units of enzyme with 0.03 µg of [$^{32}P$]lambda DNA for 1 hour at 37°C resulted in a slope of ≤1.0 slope of %-end label released per unit of enzyme. Reaction volume of 50 µl.

**DNase, single-stranded:** Incubation of 10, 20, and 40 units of enzyme with 0.03 µg of [$^{32}P$]lambda single-stranded DNA for 1 hour at 37°C resulted in a slope of ≤1.0 slope %-end label released per unit of enzyme. Reaction volume of 50 µl.

**RNase:** Incubation of 10, 20, and 40 units of enzyme with a 0.015 µg of [$^{32}P$] transcript of pPVI/PvuII for 1 hr at 37°C resulted in a slope of ≤1.0 slope %-end label released per unit of enzyme. Reaction volume of 50 µl.

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**References**

*(1) Chang, L.M. and Bollum, F.J. (1971) J. Biol. Chem. 246, 909-916 (2) Roychoudury, R. and Kossel, H. (1971) Eur. J. Biochem. 22. 310-320 (3) Tu, C.P.D. and Cohen, S.N. (1980) Gene 10, 177-183 (4) Roychoudhury, R., Jay, E. and Wu, R. (1976) Nucleic Acids Res. 3, 863-877 (5) Deng, G. and Wu, R. (1983) Methods Enzymol. 100, 96-116*