

**CERTIFICATE OF ANALYSIS**

# Terminal Deoxynucleotidyl Transferase

**#EP0161**      500 u

**Lot:**                      **Expiry Date:**

Concentration:      20 u/μl

Supplied with:      0.4 ml of 5X Reaction Buffer

**Store at -20°C**

In total 2 vials.

## Description

Terminal Deoxynucleotidyl Transferase (TdT), a template-independent DNA polymerase, catalyzes the repetitive addition of deoxyribonucleotides to the 3'-OH of oligonucleotide and single-stranded, or double-stranded DNA (1). The TdT requires an oligonucleotide of at least three nucleotides to serve as a primer.

## Source

*E. coli* cells carrying a cloned gene encoding calf thymus terminal deoxynucleotidyl transferase.

## Definition of Activity Unit

One unit of the enzyme catalyzes the incorporation of 1 nmol of deoxythymidylate into a polynucleotide fraction (adsorbed on DE-81) in 60 min at 37°C.

Enzyme activity is assayed in the following mixture: 200 mM potassium cacodylate (pH 7.2), 1 mM CoCl<sub>2</sub>, 0.1 mM DTT, 0.01% (v/v) Triton X-100, 10 μM oligo(dT)<sub>10</sub>, 1 mM dTTP and 0.4 MBq/ml [<sup>3</sup>H]-dTTP.

## Storage Buffer

The enzyme is supplied in: 100 mM potassium acetate (pH 6.8), 2 mM 2-mercaptoethanol, 0.01% (v/v) Triton X-100 and 50% (v/v) glycerol.

## 5X Reaction Buffer

1 M potassium cacodylate, 0.125 M Tris, 0.05% (v/v) Triton X-100, 5 mM CoCl<sub>2</sub> (pH 7.2 at 25°C).

## Applications

- Production of synthetic homo- and heteropolymers (1).
- Homopolymeric tailing of linear duplex DNA with any type of 3'-OH terminus (2, 3), see protocol on back page.
- Oligodeoxynucleotide and DNA labeling (2, 4-8), see protocol on back page.
- 5'-RACE (Rapid Amplification of cDNA Ends) (9).
- *In situ* localization of apoptosis (10).

## Inhibition and Inactivation

- Inhibitors: metal chelators, ammonium, chloride, iodide, phosphate ions.
- Inactivated by heating at 70°C for 10 min or by addition of EDTA.

## License

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**DE-81** – Whatman anion exchange chromatography paper having diethylaminoethyl functional groups.

**Whatman** is a registered trademark of Whatman Ltd.

**Triton X-100** is a registered trademark of Rohm & Haas, Inc.

## QUALITY CONTROL ASSAY DATA

### Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 60 units of Terminal Deoxynucleotidyl Transferase with 1 µg of pBR322 DNA in 50 µl of reaction buffer for 4 hours at 37°C.

### Exodeoxyribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 60 units of Terminal Deoxynucleotidyl Transferase with 1 µg of sonicated *E. coli* [<sup>3</sup>H]-DNA in 50 µl of reaction buffer for 4 hours at 37°C.

### Ribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 60 units of Terminal Deoxynucleotidyl Transferase with 1 µg of [<sup>3</sup>H]-RNA in 50 µl of reaction buffer for 4 hours at 37°C.

### Labeled Oligonucleotide (LO) Assay

No detectable degradation of a single-stranded and double-stranded labeled oligonucleotide was observed after incubation with 30 units of Terminal Deoxynucleotidyl Transferase for 4 hours at 37°C.

Quality authorized by:



Jurgita Zilinskiene

(continued on back page)

## Protocol for DNA Tailing

① Prepare the following reaction mixture:

<b>5X reaction buffer for Terminal Deoxynucleotidyl Transferase</b>	4 $\mu$ l
<b>DNA fragments</b>	1 pmol of 3'-ends
<b>dATP or dTTP</b> <i>or</i> <b>dGTP or dCTP</b>	130 pmol 60 pmol
<b>Terminal Deoxynucleotidyl Transferase</b>	1.5 $\mu$ l (30 u)
<b>Water, nuclease-free (#R0581)</b>	to 20 $\mu$ l

② Incubate the mixture at 37°C for 15 minutes.

③ Stop the reaction by heating at 70°C for 10 min or by the addition of 2  $\mu$ l 0.5 M EDTA (#R1021).

### **Note**

Under the conditions described above, 100-130 dA or dT residues, or 20-30 dC or dG residues per 3'-OH end of DNA can be added.

## Protocol for Labeling DNA 3'-termini

① Prepare the following reaction mixture:

<b>5X reaction buffer for Terminal Deoxynucleotidyl Transferase</b>	10 $\mu$ l
<b>DNA fragments</b>	10 pmol of 3'-termini
<b>[<math>\alpha</math>-<sup>32</sup>P]-ddATP, ~10TBq/mmol (3000Ci/mmol)</b>	1.85 MBq (50 $\mu$ Ci)
<b>Terminal Deoxynucleotidyl Transferase</b>	2 $\mu$ l (40 u)
<b>Water, nuclease-free (#R0581)</b>	to 50 $\mu$ l

② Incubate the mixture at 37°C for 15 minutes.

③ Stop the reaction by heating at 70°C for 10 min or by the addition of 5  $\mu$ l 0.5M EDTA (#R1021).

### **Note**

The efficiency of the reaction depends upon the type of 3'-OH termini of the DNA fragments. 3'-protruding ends are labeled with higher efficiency than recessed or blunt ends.

## References

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## SAFETY INFORMATION



### 5X Reaction Buffer for TDT

T Toxic

Hazard-determining components of labeling:

**cobalt dichloride**

#### Risk phrases

R49 May cause cancer by inhalation.

#### Safety phrases

S53 Avoid exposure - obtain special instructions before use.

S20 When using do not eat or drink.

S23 Do not breathe gas/fumes/vapour/spray.

S36/39 Wear suitable protective clothing and eye/face protection.

S45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

S60 This material and its container must be disposed of as hazardous waste.

#### **PRODUCT USE LIMITATION.**

This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

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