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PRODUCT INFORMATION

Terminal Deoxynucleotidyl Transferase

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Lot: _ **Expiry Date: _**

Store at -20 °C

Components	#EP0161	#EP0162
Terminal Deoxynucleotidyl Transferase, 20 U/µL	500 U	2500 U
5X Reaction Buffer	0.4 mL	2 × 1 mL

DANGER

Toxic if inhaled. May cause cancer. Toxic to aquatic life with long lasting effects. Avoid breathing dust/fume/gas/mist/vapours/spray. Use personal protective equipment as required. IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. Dispose of contents/container in accordance with local/regional/national/international regulations.

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Description

Terminal Deoxynucleotidyl Transferase (TdT), a template-independent DNA polymerase, catalyzes the repetitive addition of deoxyribonucleotides to the 3'-OH of oligodeoxyribonucleotides and single-stranded, or double-stranded DNA (1). The TdT requires an oligonucleotide of at least three nucleotides to serve as a primer. With RNA as template TdT shows variable performance which strongly depends upon the tertiary structure of acceptor RNA 3'-end and the nature of nucleotide. Generally, it is lower than using DNA as a template.

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.
- Oligodeoxyribonucleotide 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).

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 in 60 min at 37 °C.

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 $CoCl_2$ (pH 7.2 at 25 °C).

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.

Note

Due to the presence of $CoCl_2$ the TdT Reaction Buffer is incompatible with downstream applications. It is necessary to remove $CoCl_2$ from the reaction mixture by spin column or phenol/chloroform extraction and subsequent ethanol precipitation.

CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay

No detectable degradation was observed after incubation of supercoiled plasmid DNA with Terminal Deoxynucleotidyl Transferase.

Ribonuclease Assay

No detectable degradation was observed after incubation of [3H]-RNA with Terminal Deoxynucleotidyl Transferase.

Labeled Oligonucleotide (LO) Assay

No detectable degradation after incubation of single stranded and double stranded radiolabeled oligonucleotides with Terminal Deoxynucleotidyl Transferase.

Quality authorized by:  Jurgita Zilinskiene

(continued on back page)

Protocol for tailing of DNA 3’-termini

1. Prepare the following reaction mixture:

5X reaction buffer for Terminal Deoxynucleotidyl Transferase	4 µL
DNA fragments	1 pmol of 3'-ends
dATP or dTTP *or* dGTP or dCTP	130 pmol *or* 60 pmol
Terminal Deoxynucleotidyl Transferase	1.5 µL (30 U)
Water, nuclease-free (#R0581)	to 20 µL

2. Incubate the mixture at 37 °C for 15 min.
3. Stop the reaction by heating at 70 °C for 10 min or by the addition of 2 µL 0.5 M EDTA (#R1021).

Note

- Under the conditions described above, 100-130 dA or dT residues, or 20-30 dC or dG residues can be added per 3'-OH end of DNA.
- The efficiency of the reaction depends upon the type of 3’-OH termini of the DNA fragments. 3’-overhangs are tailed with higher efficiency than recessed or blunt ends.

Protocol for DNA and oligonucleotide 3’-end labeling by tailing

1. Prepare the following reaction mixture:

5X reaction buffer for TdT	10 µL
Linear DNA	10 pmol
[α-^{32}P]-ddATP, ~10 TBq/mmol (3000 Ci/mmol)	1.85 MBq (50 µCi)
Terminal Deoxynucleotidyl Transferase	2 µL (40 U)
Water, nuclease-free (#R0581)	to 50 µL

2. Incubate the mixture at 37 °C for 15 min.
3. Stop the reaction by heating at 70 °C for 10 min or by adding 5 µL 0.5 M 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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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.

Please refer www.thermofisher.com for Material Safety Data Sheet of the product.