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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

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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, 2). 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 (3). 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 (4, 5), *see* protocol on back page.
- Oligonucleotide, DNA, and RNA labeling (4, 6-11), *see* protocol on back page.
- 5'-RACE (Rapid Amplification of cDNA Ends) (12).
- *In situ* localization of apoptosis (13, 14).

Rev.14

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, heparin, chemically modified DNA.
- 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 for *N*-long tail:

5X reaction buffer for Terminal Deoxynucleotidyl Transferase	4 µL
DNA fragments	1 pmol of 3'-ends
dATP or dTTP **dGTP or dCTP**	~ 1.1*N* pmol ~ 2*N* pmol
Terminal Deoxynucleotidyl Transferase	25-40 U
Water, nuclease-free (#R0581)	to 20 µL

2. Incubate the mixture at 22-37 °C for 15-30 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

- 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.
- The highest transformation efficiency is obtained when complementary tail lengths are ~100 nt dA/T or ~20 nt dG/C.
- More uniform tail lenght distribution is achieved if reaction is performed at 22 °C with more TdT.

Protocol for DNA and oligonucleotide 3'-end labeling

1. Prepare the following reaction mixture:

5X reaction buffer for TdT	10 µL
Linear DNA	10 pmol
Radiolabelled ddATP (10 TBq / mmol)	1-2 MBq
Terminal Deoxynucleotidyl Transferase	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 exact amount of radiolabelled ddATP is determined by required specific activity of the probe, typically 1-2 MBq.
- 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. When RNA is used as the initiator for labelling, the reaction is initiated with a lower efficiency.
- Nucleotides with foreign pentose (e.g. arabinose), with modified 3'-OH groups and halogenated dNTPs are not recognized by TdT.

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