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Terminal deoxynucleotidyl Transferase, Recombinant

Cat. Nos. TDT117500, TDT11725K

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1. Introduction

Terminal deoxynucleotidyl Transferase (TdT) catalyzes the addition of mononucleotides to the 3′-OH terminus of DNA molecules. Unlike all of the other known DNA polymerases, TdT-catalyzed DNA synthesis is not template-directed. Only one dNTP is needed for polymerization, although any dNTP (and derivative) or any combination of dNTPs will serve as substrate. Protruding, recessed, or blunt-ended ssDNA or dsDNA molecules serve as a substrate. The 58.3 kDa enzyme does not have 5′- or 3′-exonuclease activity. The addition of Co^{2+} makes tailing more efficient.

The enzyme is available in 500 and 2,500-unit sizes at a concentration of 20 U/µl. The enzyme is supplied with a 10X Reaction Buffer and 2.5 mM $CoCl_2$.

2. Product Specifications

Storage: Store only at –20°C in a freezer without a defrost cycle.

Storage Buffer: 50% glycerol containing 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 0.1 mM EDTA, 1 mM DTT, and 0.1% Triton® X-100.

Unit Definition: 1 Unit is the amount of enzyme required to incorporate 1 nmol of dATP into an acid-insoluble material in 1 hour at 37°C using $d(A)_{18}$ DNA oligo as a primer.

Quality Control: TdT is function tested in a 10 µl reaction containing 33 mM Tris acetate (pH 7.5), 66 mM potassium acetate,10 mM magnesium acetate, 0.5 mM DTT, 1 mM dATP, 0.25 mM $CoCl_2$ (or acetate), 2 µM 20-mer DNA oligo and varying amounts of enzyme.

Source: An *E. coli* strain that carries the cloned TdT gene from calf thymus.

TdT 10X Reaction Buffer: 330 mM Tris-acetate (pH 7.5), 660 mM potassium acetate, 100 mM magnesium acetate, and 5 mM DTT.

Contaminating Activity Assays: TdT is free of detectable exo- and endonuclease and RNase activities.

3. Related Products:

- Fast-Link™ DNA Ligation Kit
- 2′-Deoxyribonucleoside-5′-Triphosphate Solutions

4. General Considerations

1. Inhibitors: TdT is inhibited by metal chelators (EDTA), sodium, ammonium, chloride, iodide, phosphate ions, and TRIS buffer. Due to the presence of $CoCl_2$, the reaction buffer is incompatible with downstream applications. It must be removed from the reaction mixture by precipitation of DNA with potassium acetate and ethanol, washing in 70% ethanol, and dissolving in distilled water.
2. Inactivation: Enzyme may be inactivated by heating at 70°C for 10 minutes or adding EDTA

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