



Terminal Transferase

F-203

15 000 U/ml

Store at -20°C

Stable for one year from the assay date

Description: Terminal transferase (TdT) is a template independent polymerase that catalyzes the addition of deoxynucleotides to the 3' hydroxyl terminus of DNA molecules. Protruding, recessed or blunt ended double or single stranded DNA molecules serve as a substrate for TdT.

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

Storage buffer:

60 mM KPO₄
150 mM KCl
1 mM β-mercaptoethanol
1 % Triton X-100
50 % glycerol
pH 7.2 (20°C)

1x Reaction buffer:

50 mM Potassium acetate
20 mM Tris-acetate pH 7.9 (20°C)

Enzyme is supplied with optimized 10x TdT buffer (F-203B, **does not contain CoCl₂**) and 25 mM CoCl₂ separately. To obtain 1 ml 1x assay buffer supplement with 1.5 mM CoCl₂, mix 100 µl 10x buffer and 60 µl 25 mM CoCl₂ with 840 µl H₂O.

Unit definition: One unit is defined as the amount of enzyme catalyzing the incorporation of 1 nmol dATP into acid-precipitable material in one hour at 37°C in activity assay conditions in 1 ml volume, using d(A)₁₈ as primer.

Activity assay conditions: 200 mM sodium cacodylate, 25 mM Tris-HCl pH 7.2, 8 mM MgCl₂, 0.33 mM ZnSO₄, 0.2 mM dATP; 42 pmol oligo d(A)₁₈ and 1 µCi ³H dATP (1-0.4 µM) in a 50 µl total reaction volume.

Exonuclease activity: Incubation of 50 U for 4 hours at 37°C in 50 µl assay buffer with 1 µg sonicated ³H DNA (2x10⁵ cpm/µg) released <0.5 % of radioactivity.

Endonuclease contamination: Incubation of 50 U with 1 µg φX174 RFI DNA (4 hours, 37°C, 50 µl) gave <10 % conversion to RFI.



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