Terminal Transferase

1-800-632-7799
info@neb.com
www.neb.com

M0315S 010120414041

M0315S

RR 37° Yes

500 units	**20,000 U/ml**	**Lot: 0101204**
RECOMBINANT	**Store at –20°C**	**Exp: 4/14**

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. The 58.3 KDa enzyme does not have 5´ or 3´ exonuclease activity. The addition of Co^{2+} in the reaction makes tailing more efficient.

New Reaction Buffer

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

Applications:

- Addition of homopolymer tails to the 3´ ends of DNA
- Labeling the 3´ ends of DNA with modified nucleotides (e.g., ddNTP, DIG-dUTP)
- TUNEL asay (*in situ* localization of apoptosis)
- TdT dependent PCR

Supplied in: 50 mM $KP0_4$ (pH 7.3 @ 25°C), 100 mM NaCl, 1.43 mM 2-mercaptoethanol, 0.1% Triton X-100 and 50% glycerol.

Reagents Supplied with Enzyme:
10X Terminal Transferase Reaction Buffer, 10X (2.5 mM) solution of $CoCl_2$.

Reaction Conditions: 1X Terminal Transferase Reaction Buffer, supplemented with 0.25 mM $CoCl_2$. Incubate at 37°C.

1X Terminal Transferase Reaction Buffer:
50 mM potassium acetate
20 mM Tris-acetate
10 mM magnesium acetate
pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme catalyzing the incorporation of 1 nmol dATP into acid-insoluble material in a total reaction volume of 1 ml in 1 hour at 37°C using $d(A)_{18}$ as a primer.

Unit Assay Conditions: 1X Terminal Transferase Reaction Buffer, 0.72 µM $d(A)_{18}$, 0.2 mM dATP and 1.0 µCi [^{3}H]- dATP in a 50 µl total reaction volume.

Quality Control Assays

Exonuclease Activity: Incubation of 50 units of enzyme with 1 µg sonicated [^{3}H] DNA (2×10^5 cpm/µg) for 4 hours at 37°C in 50 µl assay buffer released < 0.5% radioactivity.

Endonuclease Activity: Incubation of 50 units of enzyme with 1 µg ϕX174 RF I DNA for 4 hours at 37°C in a 50 µl reaction buffer resulted in < 10% conversion to RF II.

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

Heat Inactivation: 75°C for 20 minutes.

A Typical DNA Tailing Reaction:

1. Mix:
   a. 5.0 µl 10X TdT Buffer
   b. 5.0 µl 2.5 mM $CoCl_2$ solution provided
   c. 5.0 pmols DNA (330 ng for 100 bp, 1 µg for 300 bp, 10 pmols DNA ends)*
   d. 0.5 µl 10 mM dNTP (alpha-^{32}P dATP may also be used)
   e. 0.5 µl Terminal Transferase (20 units/µl) deionized H_20 to a final volume of 50 µl.
2. Incubate at 37°C for 30 minutes.
3. Stop the reaction by heating to 70°C for 10 minutes or by adding 10 µl of 0.2 M EDTA (pH 8.0).

*To determine approximate amount of DNA (ng/pmol), multiply the number of base pairs by 0.66. Example: 300 bp x 0.66 = 198 ng/pmol. For 5.0 pmols multiply by 5, resulting in 990 ng/5 pmol.

The table on the reverse side can be used as a guide (values are approximate and are given for a 30 minutes incubation at 37°C in the recommended buffer).

(see other side)

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The rate of addition of dNTP's and thus the length of the tail is a function of the ratio of 3´ DNA ends: dNTP concentration, and also which dNTP is used.

DNA Tailing Guide:

pmols 3´ ends pmol dNTP	Tail Length			
	dA	dC	dG	dT
1:100	1–5	1–3	1–3	1–5
1:1,000	10–20	10–20	5–10	10–20
1:5,000	100–300	50–200	10–25	200–300

References:

1. Chang, L.M. and Bollum, F.J. (1986) *CRC Crit. Rev. Biochem.* 21, 27–52.
2. Roychoudhury, R., Jay, E. and Wu R. (1976) *Nucl. Acids Res.* 3, 101–116.
3. Tu, C.-P.D. and Cohen, S.N. (1980) *Gene* 10, 177–183.
4. Boule, J.B., Rougeon, F. and Papanicolaou C. (2001) *J. Biol. Chem.* 276, 31388–31393.

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