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 assay (in situ localization of apoptosis)
- TdT dependent PCR

Reagents Supplied with Enzyme:
10X Terminal Transferase Reaction Buffer, 10X (2.5 mM) solution of 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(Adp)$_3$ as a primer.

Unit Assay Conditions: 1X Terminal Transferase Reaction Buffer, 0.72 µM d(Adp)$_3$, 0.2 mM dATP and 1.0 µCi [3H]-dATP in a 50 µl total reaction volume.

Quality Control Assays
Exonuclease Activity: Incubation of 50 units of enzyme with 1 µg sonicated [3H] DNA (2 x 10$^6$ 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 X174 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-32P dATP may also be used)
   e. 0.5 µl Terminal Transferase (20 units/µl) deionized H$_2$O 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).
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:

<table>
<thead>
<tr>
<th>pmol 3' ends pmol dNTP</th>
<th>Tail Length</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dA</td>
</tr>
<tr>
<td>1:100</td>
<td>1–5</td>
</tr>
<tr>
<td>1:1,000</td>
<td>10–20</td>
</tr>
<tr>
<td>1:5,000</td>
<td>100–300</td>
</tr>
</tbody>
</table>

## References: