**Terminal Transferase**

Object: 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 CoCl₂
- 10X Terminal Transferase Reaction Buffer, supplemented with 0.25 mM CoCl₂, 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

**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 CoCl₂ in the reaction makes tailing more efficient.

**Applications:**
1. **Addition of homopolymer tails to the 3’ ends of DNA**
2. **Labeling the 3’ ends of DNA with modified nucleotides (e.g., ddNTP, DIG-dUTP)**
3. **TUNEL assay (in situ localization of apoptosis)**
4. **TdT dependent PCR**

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

**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)₅₀ as a primer.

**A Typical DNA Tailing Reaction:**
1. **Mix:**
   - 5.0 µl 10X TdT Buffer
   - 5.0 µl 2.5 mM CoCl₂ solution provided
   - 5.0 pmols DNA (330 ng for 100 bp, 1 µg for 300 bp, 10 pmols DNA ends)*
   - 0.5 µl 10 mM dNTP (alpha-³²P dATP may also be used)
   - 0.5 µl Terminal Transferase (20 units/µl) denoized H₂O 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/µmol), multiply the number of base pairs by 0.66. Example: 300 bp x 0.66 = 198 ng/µmol. For 5.0 pmols multiply by 5, resulting in 990 ng/5 µmol.

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>pmols 3’ ends pmol dNTP</th>
<th>dA</th>
<th>dC</th>
<th>dG</th>
<th>dT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:100</td>
<td>1–5</td>
<td>1–3</td>
<td>1–3</td>
<td>1–5</td>
</tr>
<tr>
<td>1:1,000</td>
<td>10–20</td>
<td>10–20</td>
<td>5–10</td>
<td>10–20</td>
</tr>
<tr>
<td>1:5,000</td>
<td>100–300</td>
<td>50–200</td>
<td>10–25</td>
<td>200–300</td>
</tr>
</tbody>
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References: