**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

**Unit Assay Conditions:**
1X Terminal Transferase Reaction Buffer, 0.72 µM d(A)₁₈, 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 10⁵ 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.

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**A Typical DNA Tailing Reaction:**

1. **Mix:**
   - a. 5.0 µl 10X TdT Buffer
   - b. 5.0 µl 2.5 mM CoCl₂ solution provided
   - c. 5.0 pmoles DNA (330 ng for 100 bp, 1 µg for 300 bp, 10 pmoles 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)
   - f. 0.1 µCi [³H]-dATP in a 50 µl total reaction volume.

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).**

4. The solution 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>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: