Terminal Transferase

**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

**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 Co²⁺ makes tailing more efficient.

**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 [³²P]-dATP in a 50 µl total reaction volume.

**Quality Control Assays**

**Exonuclease Activity:** Incubation of 50 units of enzyme with 1 µg sonicated [³²P]-DNA (2 x 10⁶ 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 pX174 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:**
   - 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 (α³²P-dATP may also be used)
   - 0.5 µl Terminal Transferase (20 units/µl) deionized 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/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.

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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).

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**New Reaction Buffer**

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**New Reaction 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 dC dG dT</td>
</tr>
<tr>
<td>1:100</td>
<td>1–5 1–3 1–3 1–5</td>
</tr>
<tr>
<td>1:1,000</td>
<td>10–20 10–20 5–10 10–20</td>
</tr>
<tr>
<td>1:5,000</td>
<td>100–300 50–200 10–25 200–300</td>
</tr>
</tbody>
</table>

**References:**