**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 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 [3H]-dATP in a 50 µl total reaction volume.

**Exonuclease Activity:** Incubation of 50 units of enzyme with 1 µg sonicated [3H] 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 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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**Terminal Transferase**

**Description:** Terminal Transferase (TdT) is a template independent polymerase that catalyzes the addition of deoxynucleotides to the 3’ hydroxyl terminus of DNA molecules. Promoting, 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+ in the reaction makes tailing more efficient.

**Reagents Supplied with Enzyme:**
- 10X Terminal Transferase Reaction Buffer
- 10X (2.5 mM) solution of CoCl2

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

**Quality Control Assays**

**Heat Inactivation:**
- 75°C for 20 minutes.

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**A Typical DNA Tailing Reaction:**

**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) deionized H2O to a final volume of 50 µl

1. Incubate at 37°C for 30 minutes.
2. Stop the reaction by heating to 70°C for 10 minutes or by adding 10 µl of 0.2 M EDTA (pH 8.0).

**TnDNA Tailing Reaction:**

**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) deionized H2O to a final volume of 50 µl

1. Incubate at 37°C for 30 minutes.
2. Stop the reaction by heating to 70°C for 10 minutes or by adding 10 µl of 0.2 M EDTA (pH 8.0).

**Quality Control Assays**

**Heat Inactivation:**
- 75°C for 20 minutes.
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</th>
<th>Tail Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>pmol dNTP</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:


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