Product Information

Terminal Deoxynucleotidyl Transferase (TdT)

Catalog Number KEM0032
Storage Temperature –20 °C
Unit Size 6,000 U

Product Description
Terminal deoxynucleotidyl transferase (TdT) is a template-independent DNA polymerase that catalyzes the addition of deoxynucleotides to the 3' hydroxyl terminus of single or double stranded DNA molecules. The presence of 1 mM Co²⁺ stimulates the tailing of the 3'-ends of DNA fragments. This construct is sold as an N-terminal truncation of the terminal transferase gene attached to an N-terminal fusion tag.

Source of Protein
An E. coli strain that carries the cloned terminal transferase gene from calf thymus.

Reagent
Supplied at a concentration 20,000 U/mL in 50 mM KPO₄, 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 0.1% Triton™ X-100, 50% glycerol, pH 7.3

Supplied with:
2.5 mM CoCl₂
Catalog Number KEM0045B

10X Green Buffer
Catalog Number KEM0043B
200 mM Tris-Acetate, 500 mM potassium acetate, 100 mM magnesium acetate, 10 mM DTT, pH 7.9

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Unit Definition
1 unit is defined as the amount of polymerase required to convert 1 nmol of dTTPs into acid insoluble material in 1 hour at 37°C.

Protocol Reaction setup:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (µL)</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X Green Buffer</td>
<td>5 µL</td>
<td>1X</td>
</tr>
<tr>
<td>10 pmol DNA termini (10-100ng)</td>
<td>X µL</td>
<td>1-10 ng/µL</td>
</tr>
<tr>
<td>Deoxynucleotide solution</td>
<td>X µL</td>
<td>200 µM</td>
</tr>
<tr>
<td>Terminal Transferase (20 U/µL)</td>
<td>1 µL</td>
<td>0.4 U/µL</td>
</tr>
<tr>
<td>Sterile Water</td>
<td>X µL</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Total Volume</strong></td>
<td><strong>50 µL</strong></td>
<td></td>
</tr>
</tbody>
</table>

*Total reaction volume can be adjusted as needed.

1. Incubate at 37 °C for 30 minutes.
2. Inactivate the TdT and stop the reaction by heating to 70 °C for 10 minutes.

Usage Notes:
1. Co²⁺ increases the nucleotide incorporation efficiency of pyrimidines, and at blunt and 3’ recessed ends. However, the addition of dNTPs to 3’-overhanging ends is more efficient than with 3’-recessed or blunt ends. TdT requires a free 3’-hydroxyl group in order to make a non-templated nucleotide addition.
2. With limited efficiency, Terminal Transferase will incorporate ribonucleotides, biotinylated, and dideoxynucleotides in the presence of Co²⁺.
3. Terminal Transferase incorporates dATP and dTTP with a 5-fold higher efficiency than dCTP and dGTP, as evidenced by the following Kₘ values for nucleotides:

<table>
<thead>
<tr>
<th>Base</th>
<th>Km</th>
<th>Base</th>
<th>Km</th>
</tr>
</thead>
<tbody>
<tr>
<td>dATP</td>
<td>100 µM</td>
<td>dCTP</td>
<td>500 µM</td>
</tr>
<tr>
<td>dTTP</td>
<td>100 µM</td>
<td>dGTP</td>
<td>500 µM</td>
</tr>
</tbody>
</table>

References:

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