**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.

**Supplied in**
- 50 mM KPO₄
- 100 mM NaCl
- 1.0 mM DTT
- 0.1mM EDTA
- 0.1% Triton X-100
- 50% Glycerol
- pH 7.3 @ 25°C

**Supplied With**
- B0120 (10X Green Buffer)
- B0220 (2.5 mM CoCl₂)

**10X Green Buffer (B0120):**
- 200 mM Tris-Acetate
- 500 mM Potassium Acetate
- 100 mM Magnesium Acetate
- 10 mM DTT
- pH 7.9 @ 25°C

**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.

**Quality Control Analysis:**

**Unit Characterization Assay**
Unit activity was measured using a 2-fold serial dilution method. Dilutions of enzyme were made in 1X reaction buffer and added to 50 µL reactions containing Oligo dT 20mer DNA, 1X reaction buffer, 0.25mM CoCl₂, ³H-dTTP and 100 µM dTTPs. Reactions were incubated 10 minutes at 37°C, plunged on ice, and analyzed using the method of Sambrook and Russell (*Molecular Cloning, v3, 2001, pp. A8.25-A8.26*).

**Protein Concentration (OD₂₈₀) Measurement**
A 2.0 µL sample of TdT was analyzed at OD₂₈₀ using a Nanodrop ND-1000 spectrophotometer standardized using a 2.0 mg/ml BSA sample (Pierce Cat #23209 and blanked with TdT storage solution). The observed average measurement of 3 replicate samples was converted to mg/mL using an extinction coefficient of 88,190 and molecular weight of 82,598 Daltons.

**SDS-Page (Physical Purity Assessment)**
2.0 µL of concentrated enzyme solution was loaded on a denaturing 4-20% Tris-Glycine SDS-PAGE gel flanked by a broad-range MW marker and 2.0 µL of a 1:100 dilution of the sample. The acceptance criteria for this test requires that the aggregate mass of contaminant bands in the concentrated sample do not exceed the mass of the protein of interest band in the dilute sample, confirming greater than 99% purity of the concentrated sample.
**Contamination Tests:**

**Single-Stranded Exonuclease Activity**
A 50 µl reaction containing 10,000 cpm of a radiolabeled single-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C resulted in less than 5.0% release of TCA-soluble counts.

**Double-Stranded Exonuclease Activity**
A 50 µl reaction containing 5,000 cpm of a radiolabeled double-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C resulted in less than 1.0% release of TCA-soluble counts.

**Double-Stranded Endonuclease Activity**
A 50 µl reaction containing 0.5 µg of pENZuC DNA and 10 µL of enzyme solution incubated for 4 hours at 37°C resulted in no visually discernible conversion to nicked circular DNA as determined by agarose gel electrophoresis.

**E.coli 16S rDNA Contamination Test**
Replicate 5 µL samples of enzyme solution were denatured and screened in a TaqMan qPCR assay for the presence of contaminating E.coli genomic DNA using oligonucleotide primers corresponding to the 16S rRNA locus. The acceptance criterion for the test is the threshold cycle count (Ct) produced by the average of 3 replicate no template control samples. Based on the correlation between the no template control Ct values, and standard curve data, the detection limit of this assay is <10 copies genome/sample.

**Notes:**
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.

With limited efficiency, Terminal Transferase will incorporate ribonucleotides, biotinylated, and dideoxynucleotides in the presence of Co²⁺.

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

**Usage Instructions:**
Non-templated addition of dNTPs to 3’ termini of DNA:

**Reaction Set-Up:**

<table>
<thead>
<tr>
<th>Amount</th>
<th>Description</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 µL</td>
<td>10X Green Buffer (B012)</td>
<td>1X</td>
</tr>
<tr>
<td>X µL</td>
<td>10 pmol DNA termini (10-100 ng)</td>
<td>1-10 ng/µL</td>
</tr>
<tr>
<td>X µL</td>
<td>Deoxynucleotide solution</td>
<td>200 µM</td>
</tr>
<tr>
<td>1 µL</td>
<td>Terminal Transferase (20 U/ µL)</td>
<td>30 U/µL</td>
</tr>
<tr>
<td>X µL</td>
<td>Type I Water</td>
<td>N/A</td>
</tr>
<tr>
<td>50 µL</td>
<td>Total Volume</td>
<td></td>
</tr>
</tbody>
</table>

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

**References:**

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**Limitations of Use**
This product was developed, manufactured, and sold for *in vitro* use only. The product is not suitable for administration to humans or animals. MSDS sheets relevant to this product are available upon request.