Terminal Deoxynucleotidyl Transferase, Recombinant:

Part No.       Size (units)
M826A          300
M828C          1,500

Description: This enzyme catalyzes the repetitive addition of mononucleotides to the terminal 3’-OH of a DNA initiator accompanied by the release of inorganic phosphate. Single-stranded DNA is preferred as an initiator. Polymerization is not template-dependent. The addition of 1mM CoCl2 (as CoCl2) in the reaction buffer allows the tailing of 3’-ends with varying degrees of efficiency.

Enzyme Storage Buffer: Terminal Deoxynucleotidyl Transferase, Recombinant, is supplied in 50mM potassium phosphate (pH 6.4), 100mM NaCl, 1mM β-mercaptoethanol, 0.1% Tween® 20 and 50% glycerol.

Source: Recombinant E. coli strain.

Storage Conditions: See the Product Information Label for storage recommendations. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. See the expiration date on the Product Information Label.

Terminal Transferase 5X Buffer (M189A): 500mM cacodylate buffer (pH 6.8), 5mM CoCl2 and 0.5mM DTT.

Unit Definition: One unit of activity catalyzes the transfer of 0.5 picomoles of ddATP to oligo(dT)16 per minute at 37°C in 1X Terminal Transferase Buffer. The resulting oligo(dT)17 is measured by HPLC.

Usage Notes for 3’-End Labeling Reaction
1. Not all dNTPs are tailed with the same efficiency. Actual concentration of dNTP will depend on the individual application.
2. The provided buffer (5X) is to be used in the tailing reaction. The recommended reaction conditions are as described under Quality Control Assays, 3’-End Labeling Reaction, and in Section III overleaf.

Quality Control Assays

Functional Assays

3’-End Labeling Reaction: Two micromolar oligo(dT)16 is incubated in 1X Terminal Transferase Buffer together with 40µM ddATP and 1 unit of Terminal Deoxynucleotidyl Transferase (in a final volume of 50µl) for 30 minutes at 37°C. The specification is that >50% of the oligo(dT)16 is converted to oligo(dT)17.

TUNEL Assay: HL-60 cells at a concentration of 5 × 10^5 cells/ml are treated with anisomycin in DMSO and incubated at 37°C for 2 hours. The cells are fixed onto microscope slides and processed for TUNEL staining using 30 units of Terminal Deoxynucleotidyl Transferase, Recombinant, and the DeadEnd™ Fluorometric TUNEL System (Cat. # G3250). The procedure is outlined in Technical Bulletin #TB235 (www.promega.com/tbs/tb235/tb235.html)

Contaminant Activity

DNase and RNase Assay: To test for nuclease activity, 50ng of radiolabeled DNA or radiolabeled RNA is incubated with 25 units of Terminal Deoxynucleotidyl Transferase, Recombinant, in 1X Reaction Buffer plus 10mM MgCl2 for one hour at 37°C, and the release of radiolabeled nucleotides is monitored by scintillation counting of TCA-soluble material. The specification is <3% release for both DNase and RNase.

Endonuclease Assay: To test for endonuclease activity, 1µg of Type I supercoiled plasmid DNA is incubated with 5 units of Terminal Deoxynucleotidyl Transferase, Recombinant, in 1X Reaction Buffer plus 10mM MgCl2 for one hour at 37°C. Following incubation, the supercoiled DNA is visualized on an ethidium bromide-stained agarose gel to verify the absence of visible nicking or cutting.

Signed by: R. Wheeler, Quality Assurance
I. Description
Terminal Deoxynucleotidyl Transferase, Recombinant (rTdT), is an enzyme that catalyzes the repetitive addition of mononucleotides from dNTPs to the terminal 3'-OH of a DNA initiator, accompanied by the release of inorganic phosphate (1). The enzyme thus provides a unique method for the labeling of the 3’ termini of DNA.

II. Product Components

<table>
<thead>
<tr>
<th>Product</th>
<th>Size</th>
<th>Cat.#</th>
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</thead>
<tbody>
<tr>
<td>Terminal Deoxynucleotidyl Transferase, Recombinant</td>
<td>300 units</td>
<td>M1871</td>
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<tr>
<td></td>
<td>1,500 units</td>
<td>M1875</td>
</tr>
</tbody>
</table>

In addition to Terminal Deoxynucleotidyl Transferase, M1871 and M1875 also include:

- 500µl Terminal Transferase 5X Buffer

III. Addition of $[\alpha-^{32}P]dNTP$ to the 3’ Termini of Single-Stranded DNA Primers

1. Set up the following reaction:
   - Terminal Transferase 5X Buffer 4.0µl
   - Primer 2pmol
   - $[\alpha-^{32}P]dATP$ (800Ci/mmol, 10mCi/ml) 1.6µl
   - Terminal Deoxynucleotidyl Transferase, Recombinant 10–20 units
   - Water to a final volume of 20µl

2. Incubate at 37°C for 60 minutes.
3. Stop the reaction by heating at 70°C for 10 minutes.

<table>
<thead>
<tr>
<th>Primer Length</th>
<th>Amount (ng) of Primer Needed to Equal 2pmol</th>
</tr>
</thead>
<tbody>
<tr>
<td>15mer</td>
<td>10ng</td>
</tr>
<tr>
<td>18mer</td>
<td>12ng</td>
</tr>
<tr>
<td>24mer</td>
<td>16ng</td>
</tr>
<tr>
<td>31mer</td>
<td>21ng</td>
</tr>
</tbody>
</table>

Table 1. Amount of DNA Primer (ng) Needed to Equal 2pmol.

Notes:
1. The length and distribution of the homopolymer tails added by TdT depends on several factors including the nucleotide used, substrate concentrations, ratio of DNA primer to nucleotide and reaction time and temperature. Reference 2 contains a discussion of the factors affecting the length and distribution of homopolymer tails generated by native TdT.
2. The enzyme-to-substrate ratio is also critical for obtaining uniform addition of labeled nucleotides. Therefore, 10–20 units of enzyme are recommended for 2–4pmol of substrate DNA. Ratios lower than this produce probes of varying lengths and reduce incorporation rates.
3. Reactions can be carried out overnight without affecting the quality of the end product.
4. Enzyme activity may be inhibited if the volume of radioactive label present in the reaction exceeds 40% of the total reaction volume. To avoid this problem, reactions can be scaled up accordingly.
5. In the presence of Co2+ (present in 5X buffer), double-stranded DNA may also be labeled. DNA with a protruding 3’ terminus is preferred. Blunt-ended or recessed 3’ termini may also be labeled, although not with uniform efficiency.

IV. Determination of Percent Incorporation/Specific Activity

A. Protocol

<table>
<thead>
<tr>
<th>Materials to Be Supplied by the User</th>
</tr>
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<tbody>
<tr>
<td>0.2M EDTA</td>
</tr>
<tr>
<td>0.5M Na2HPO4 (pH 6.8)</td>
</tr>
<tr>
<td>Whatman® DE-81 2.3cm circular filters</td>
</tr>
</tbody>
</table>

1. Dilute 1µl of the reaction mixture into 100µl of 0.2M EDTA. Spot 3µl of this solution onto each of four Whatman® DE-81 2.3cm circular filters.
2. Dry the filters briefly under a heat lamp. Set two filters aside for use in determining total cpm.
3. Wash the other two filters in 50ml of 0.5M Na2HPO4 (pH 6.8) twice for 5 minutes each to remove unincorporated nucleotides.
4. Dry the washed filters under a heat lamp.
5. Add the appropriate scintillation fluid to each filter and count in a scintillation counter.

B. Example of a Standard Calculation

\[ \% \text{ incorporation} = \frac{\text{incorporated cpm}}{\text{total cpm}} \times 100 \]

Total cpm incorporated = incorporated cpm \times \text{dilution factor} \times \frac{\text{total reaction volume}}{\text{volume counted}}

Average number of bases added to each primer

\[ \frac{\% \text{ incorporation} \times \text{molar ratio of nucleotide to primer present in the reaction}}{100} \]

Amount of DNA synthesized

\[ \frac{\text{average number of bases added per primer} \times 330\text{pg/pmol base}}{\text{pmol primer present in reaction}} \]

Specific activity of probe = \frac{\text{total cpm incorporated}}{\mu\text{g of DNA template} + \mu\text{g DNA synthesized}}

V. Composition of Buffers and Solutions

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2M EDTA (pH 8.0)</td>
<td>37.22g disodium ethylene-diaminetetraacetate •2H2O</td>
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<tr>
<td></td>
<td>Add the EDTA to 300ml of water, adjust the pH to 8.0 with NaOH pellets and stir until the EDTA is in solution. Adjust the final volume to 500ml with water and filter sterilize.</td>
</tr>
<tr>
<td>0.5M Na2HPO4 (pH 6.8)</td>
<td>47.25g NaOH • Na2HPO4 (monobasic)</td>
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<td></td>
<td>22.35g Na2HPO4 (dibasic)</td>
</tr>
</tbody>
</table>

VI. References