

Product Contents

Terminal Deoxynucleotidyl Transferase, Recombinant:

Part No.	Size (units)
M828A	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 Co²⁺ (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.

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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 × 10⁵ 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.

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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

Product	Size	Cat.#
Terminal Deoxynucleotidyl Transferase, Recombinant	300 units	M1871
	1,500 units	M1875

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

- 500µl Terminal Transferase 5X Buffer

III. Addition of [α -³²P]dNTP to the 3' Termini of Single-Stranded DNA Primers

1. Set up the following reaction:

Terminal Transferase 5X Buffer	4.0µl
primer	2pmol
[α - 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 1. Amount of DNA Primer (ng) Needed to Equal 2pmol.

Primer Length	Amount (ng) of Primer Equal to 2pmol
15mer	10ng
18mer	12ng
24mer	16ng
31mer	21ng

In general, ng of primer = pmol of primer \times 0.33 \times N, where N = length of primer in bases.

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

Materials to Be Supplied by the User

(Solution compositions are provided in Section V.)

- 0.2M EDTA
- 0.5M Na₂HPO₄ (pH 6.8)
- Whatman® DE-81 2.3cm circular filters

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

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

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

(*Dilution factor is from Section IV.A, Step 1.)

Average number of bases added to each primer

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

Amount of DNA synthesized

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

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

V. Composition of Buffers and Solutions

0.2M EDTA (pH 8.0)

37.22g disodium ethylene-diaminetetraacetate • 2H₂O

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.

0.5M Na₂HPO₄ (pH 6.8)

47.25g NaH₂PO₄ (monobasic)
22.35g Na₂HPO₄ (dibasic)

Add water, slowly, to a final volume of 1 liter and filter sterilize.

VI. References

1. Kato, K. *et al.* (1967) Deoxynucleotide polymerizing enzymes of calf thymus gland. II. Properties of terminal deoxynucleotidyl transferase. *J. Biol. Chem.* **242**, 2780–9.
2. Eun, H-M. (1996) *Enzymology Primer for Recombinant DNA Technology*, Academic Press, Inc., San Diego, CA.