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Terminal Transferase (TdT)

Catalog Number: E108-1, E108-2

Table 1. Kit Components and Storage

Kit Component	E108-1 (500 units)	E108-2 (2500 units)	Storage	Stability
Terminal Transferase (20 units/µL)	25 µL	125 µL	-20 °C, avoid repeated free-thaw	The product is stable for 12 months when stored as directed.
10× Reaction Buffer	250 µL	1.25 mL		
2.5 mM $CoCl_2$	250 µL	1.25 mL		

Product Description

Terminal transferase (TdT) is a template independent polymerase that catalyzes the addition of deoxynucleotides to the 3' hydroxyl terminus of DNA molecules. Protruding, 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 Co^{2+} in the reaction makes tailing more efficient.

The enzyme is available in 500 and 2,500 unit sizes at a concentration of 20 U/µL. The enzyme is supplied with a 10× Reaction Buffer and 2.5 mM $CoCl_2$.

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 asay (in situ localization of apoptosis).
- TdT dependent PCR.

Product Specifications

- **Storage Buffer:** 50 mM potassium phosphate (pH 6.4), 100 mM NaCl, 1 mM DTT, 0.1% Triton X-100, and 50% (v/v) glycerol.
- **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 Reaction Buffer, 0.72 µM $d(A)_{18}$, 0.2 mM dATP and 1.0 µCi [^{3}H]- dATP in a 50 µl total reaction volume.

DNA Tailing Protocol

1. Assemble the following reaction at room temperature:

10× Reaction Buffer	5 µL
2.5 mM $CoCl_2$ solution	5 µL
DNA	5 pmols (330 ng for 100 bp, 1 µg for 300 bp, 10 pmols DNA ends)*
10 mM dNTP (or α-^{32}P dATP)	0.5 µL
TdT	0.5 µL
Nuclease-free water	to 50 µL

Total volume	50 µL

*To determine approximate amount of DNA (ng/pmol), multiply the number of base pairs by 0.66. Example: 300 bp x 0.66 = 198 ng/pmol. For 5.0 pmols multiply by 5, resulting in 990 ng/5 pmol.

The table below can be used as a guide (values are approximate and are given for a 30 minutes incubation at 37°C in the recommended buffer).

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:

3' ends : dNTP (mol ratio)	Tail Length			
	dA	dC	dG	dT
1:100	1-5	1-3	1-3	1-5
1:1,000	10-20	10-20	5-10	10-20
1:5,000	100-300	50-200	10-25	200-300

2. Mix gently and spin down for a few seconds.
3. Incubate at 37 °C for 30 min.
4. Stop the reaction by heating at 70°C for 10 min or adding 4 µL of 0.5 M EDTA (pH 8.0).

A-Tailing (single nucleotide) Protocol

1. Assemble the following reaction at room temperature:

10× Reaction Buffer	7.5 µL
2.5 mM $CoCl_2$ solution	7.5 µL
PCR-amplified DNA	X µL
1 mM ddATP	1.5 µL
TdT	6 µL
Nuclease-free water	to 75 µL
Total volume	75 µL

2. Mix gently and spin down for a few seconds.
3. Incubate at 37 °C for 1.5 h.