

3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Terminal Transferase from calf thymus

Catalog Number **T4427** Storage Temperature –20 °C

CAS RN 9027-67-2 EC 2.7.7.31

Synonyms: TdT: Terminal Deoxynucleotidyl

Transferase

Product Description

Terminal transferase is a unique DNA polymerase. In the presence of a divalent cation, it catalyzes the addition of deoxynucleotides to the 3′-hydroxyl terminus of DNA with the release of inorganic phosphate. The enzyme demonstrates a preference for a 3′ overhang; however, blunt or 3′ recessed ends can be used, although less effectively. Mg²+ is the preferred cation when a purine (dATP or dGTP) is to be added to DNA with a 3′ overhang. When a pyrimidine (TTP or dCTP) is to be added, Co²+ should be used. Blunt ended or 3′ recessed DNA can be more effectively tailed using Co²+ or Mn²+.1.2

These properties have proven to be useful in a number of applications including cloning and radioactive or non-radioactive labeling of DNA:^{3,5,6}

- Add homopolymers to vectors, inserts and cDNA for cloning
- Label the 3'-end of double- and single-stranded DNA with non-radioactive and radioactive labels
- Carry out in vitro mutagenesis by adding single nucleotides to DNA
- Use in TUNEL assays with deoxyuridine triphosphate (dUTP) conjugated with a fluorescent chromophore or biotin to label nicked DNA in apoptotic cells

The product is supplied as a solution in 50 mM potassium phosphate, pH 7.4, with 1 mM 2-mercaptoethanol and 50% glycerol (v/v).

Activity: ≥5,000 units/ml

Unit Definition: One unit will incorporate 1 nanomole of dATP into acid precipitable material in one hour at 37 °C using oligo (dT)₆ as a primer.

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.

Storage/Stability

The product ships on dry ice and storage at –20 °C is recommended.

Procedure

Suggested Reaction Buffers:

For purine tailing:

100 mM potassium cacodylate, pH 7.2

4 mM MqCl₂

1 mM purine (dATP or dGTP)

20-50 units of terminal transferase

1-30 pmoles of 3' ends

25-30 µl final volume

Incubate at 37 °C for 1 hour

For pyrimidine tailing:

100 mM potassium cacodylate, pH 7.2

2 mM CoCl₂

1 mM pyrimidine (TTP or dCTP)

20-50 units of terminal transferase

1-30 pmoles of 3' ends

25-3 µl final volume

Incubate at 37 °C for 1 hour

References

- Deng, G-R., and Wu, R., Methods in Enzymology, 100, 96 (1983).
- 2. Roychoudhury, R. *et al.*, Nucl. Acids Res., **3**, 863 (1976).
- 3. Sambrook, J. *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press (Cold Spring Harbor, NY: 1989) p. 5.56.
- 4. Tu, C.-P.O., and Cohen, S.N., Gene, 10, 177 (1980).
- 5. Kokym, R., and Horth, E., Int. J. Radiat. Biol., **68**, 133 (1995).
- 6. Figeys, D. et al., Anal Chem., 23, p. 4382 (1994)

JB,KH,EWK,MAM 01/07-1