3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Terminal Deoxynucleotidyl Transferase (TdT)

Catalog Number **KEM0032**
Storage Temperature –20 °C
Unit Size 6,000 U

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 Co^{2+} 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.

Reagent
Supplied at a concentration 20,000 U/mL in 50 mM KPO_4, 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 0.1% Triton™ X-100, 50% glycerol, pH 7.3

Supplied with:

2.5 mM $CoCl_2$
Catalog Number KEM0045B

10X Green Buffer
Catalog Number KEM0043B
200 mM Tris-Acetate, 500 mM potassium acetate, 100 mM magnesium acetate, 10 mM DTT, pH 7.9

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**.**

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.

Protocol Reaction setup*:

Component	Volume (µL)	Final Concentration
10X Green Buffer	5 µL	1X
10 pmol DNA termini (10-100ng) 10 1x	X µL	1-10 ng/µL
Deoxynucleotide solution	X µL	200 µM
Terminal Transferase (20 U/µL)	1 µL	0.4 U/µL
Sterile Water	X µL	N/A
Total Volume	**50 µL**	

* Total reaction volume can be adjusted as needed.

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

Usage Notes:
1. Co^{2+} 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.
2. With limited efficiency, Terminal Transferase will incorporate ribonucleotides, biotinylated, and dideoxynucleotides in the presence of Co^{2+}.
3. Terminal Transferase incorporates dATP and dTTP with a 5-fold higher efficiency than dCTP and dGTP, as evidenced by the following K_m values for nucleotides:
4.

Base	Km	Base	Km
dATP	100 µM	dCTP	500 µM
dTTP	100 µM	dGTP	500 µM

References:
1. Deng, G.R. and Wu, R., *Meth. Enxymol*. **100**, 96-116 (1983).

TJ,PHC 06/14-1