



Product Information

Terminal deoxy Transferase (TdT)

Part Number: P707L

Lot Number:

Certificate of Analysis

2010-P707-Rev.E

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.

Supplied in

100 mM KPO_4
100 mM NaCl
1.0 mM DTT
0.1% Triton X-100
50% Glycerol
pH 7.3 @ 25°C

Supplied With

10X Green Buffer (B012), 2.5 mM CoCl_2 (B022)

10X Green Buffer (B012)

200 mM Tris -Acetate
500 mM Potassium Acetate
100 mM Magnesium Acetate
10 mM DTT
pH 7.9 @ 25°C

Product Specification*

Unit Size:	6,000 Units
Unit Concentration	20,000 U/mL
Volume	0.3 mL
Purity (SDS-PAGE)	>99%
Specific Activity (est.)	27,000 U/mg
SS Exonuclease	100 U <0.1% released
DS Exonuclease	100 U <0.1% released
Endonuclease	100 U <10% converted
<i>E. coli</i> 16S rDNA Contamination	100 U <10 copies
Storage	-20°C

* For a detailed summary of assay conditions and data, refer to the Quality Controls Analysis section below

Unit Definition

1 unit is defined as the amount of polymerase required to convert 10 nmol of dNTPs into acid insoluble material in 30 minutes at 37°C.

Quality Control Analysis:

Unit Characterization Assay

Unit activity was measured using a 2-fold serial dilution method. Dilutions of enzyme were made in a glycerol (50%) containing TdT storage solution ($[\text{TdT}]_f = 0.12\text{-}0.002\mu\text{g}/\mu\text{L}$) and added to 50 μL reactions containing 0.5 pM Calf Thymus DNA, 1X Green Buffer, 4mCi/mL ^3H -dTTP and 100 μM dTTPs. Reactions were incubated 10 minutes at 37°C, plunged on ice, and analyzed using the method of Sambrook and Russell (*Molecular Cloning, v3, 2001, pp. A8.25-A8.26*).

Protein Concentration (OD_{280}) Measurement

A 2.0 μL sample of TdT was analyzed at OD_{280} using a Nanodrop ND-1000 spectrophotometer standardized using a 2.0 mg/ml BSA sample (Pierce Cat #23209 and blanked with TdT storage solution. The observed average measurement of 3 replicate samples was converted to mg/mL using an extinction coefficient of 88,190 and molecular weight of 82,598 Daltons. Acceptance for this assay is +/- 5% of reference sample.

SDS-Page (Physical Purity Assessment)

2.0 µL of concentrated enzyme solution was loaded on a denaturing 4-20% Tris-Glycine SDS-PAGE gel flanked by a broad-range MW marker and 2.0 µL of a 1:100 dilution of the sample. The acceptance criteria for this test requires that the aggregate mass of contaminant bands in the concentrated sample do not exceed the mass of the protein of interest band in the dilute sample, confirming greater than 99% purity of the concentrated sample.

Nuclease Contamination Tests:

Single-Stranded Exonuclease Activity

A 50 µl reaction containing 15,000 cpm of a radiolabeled single-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C resulted in less than 0.1% release of TCA-soluble counts.

Double-Stranded Exonuclease Activity

A 50 µl reaction containing 15,000 cpm of a radiolabeled double-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C resulted in less than 0.1% release of TCA-soluble counts.

Endonuclease Activity

A 50 µl reaction containing 1 µg of pENZuC DNA and 10 µL of enzyme solution incubated for 4 hours at 37°C resulted in no visually discernible conversion to nicked circular DNA as determined by agarose gel electrophoresis.

E. coli 16S rDNA Contamination Test

Replicate 5 µL samples of enzyme solution were heat denatured and screened in a TaqMan qPCR assay for the presence of contaminating *E. coli* genomic DNA using primers for the 16S rRNA locus. Based on no template control C_t values, the detection limit of this assay is <10 copies genome/sample.

Notes:

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.

With limited efficiency, Terminal Transferase will incorporate ribonucleotides, biotinylated, and dideoxynucleotides in the presence of Co^{2+} .

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:

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

Usage Protocol:

Non-templated addition of dNTPs to 3' termini of DNA:

Reaction Set-Up:

Amount	Description	Final Concentration
5 µL	10X Green Buffer (B012)	1X
X µL	10 pmol DNA termini (10-100 ng)	1-10 ng/µL
x µL	Deoxynucleotide solution	200 µM
1 µL	Terminal Transferase (20 U/ µL)	30 U/µL
X µL	Type I Water	N/A
50 µL	Total Volume	

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

References:

1. Deng, G.R. and Wu, R. 1983 Meth. Enzymol. 100:96-116.



Limitations of Use

This product was developed, manufactured, and sold for *in vitro* use only. The product is not suitable for administration to humans or animals. MSDS sheets relevant to this product are available upon request.