Product Information	
Terminal deoxynucleotidyl Transferase (TdT)	
Part Number	P7070L
Concentration	20,000 U/mL
Unit Size	6,000 U
Storage Temperature	-25°C to -15°C
Lot Number	
Reference Number	

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 (1,2).

Product Specifications						
P7070						
Assay	**SDS Purity**	**Specific Activity**	**SS Exonuclease**	**DS Exonuclease**	**DS Endonuclease**	***E. coli* DNA Contamination**
Units Tested	**n/a**	**n/a**	**200**	**200**	**200**	**200**
Specification	>99%	27,400 U/mg	<5.0% Released	<1.0% Released	No Conversion	<10 copies

Source of Protein: An *E. coli* strain that carries the cloned terminal transferase gene from calf thymus with an N-terminal fusion tag.

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.

Molecular weight: 82.6 kDa

Quality Control Analysis:

Unit Activity is measured using a 2-fold serial dilution method. Dilutions of enzyme were made in 1X reaction buffer and added to 50 µL reactions containing Oligo dT 20 mer DNA, 1X reaction buffer, 0.25 mM $CoCl_2$ ^{3}H-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 (3).

Protein Concentration (OD_{280}) is determined by OD_{280} absorbance.

Physical Purity is evaluated by SDS-PAGE of concentrated and diluted enzyme solutions followed by silver stain detection. Purity is assessed by comparing the aggregate mass of contaminant bands in the concentrated sample to the mass of the protein of interest band in the diluted sample.

Single-Stranded Exonuclease is determined in a 50 µL reaction containing a radiolabeled single-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.

Double-Stranded Exonuclease is determined in a 50 µL reaction containing a radiolabeled double-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.

Double-Stranded Endonuclease is determined in a 50 µL reaction containing 0.5 µg of plasmid DNA and 10 µL of enzyme solution incubated for 4 hours at 37°C.

***E. coli* 16S rDNA Contamination** is evaluated using 5 µL replicate samples of enzyme solution denatured and screened in a TaqMan qPCR assay for the presence of contaminating *E. coli* genomic DNA using oligonucleotide primers corresponding to the 16S rRNA locus.

Supplied 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 at 25°C)

Supplied with:
10X Green Buffer (B0120): 200 mM Tris-Acetate, 500 mM Potassium Acetate, 100 mM Magnesium Acetate (pH 7.9 at 25°C)
B0220: 2.5 mM $CoCl_2$

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

1. Set up the following reaction mixture in a total volume of 50 µL:

Components	Final Concentration	Volume
Type I Water	N/A	X µL
10X Green Buffer (B0120)	1X	5 µL
2.5 mM $CoCl_2$ (B0220)	250 µM	5 µL
10 pmol DNA termini (10-100 ng)	1-10 ng/µL	X µL
dNTP mix	200 µM	X µL
TdT (P7070L)	0.4 U/µL	1 µL
	Total Volume =	50 µL

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

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 to make a non-templated nucleotide addition.

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

References:
1. Deng, G.R. and Wu, R. (1983) Meth. Enzymol., 100:96-116.
2. Roychoudhury, R. et al. (1976) Nucl. Acids Res., 3,101-116.
3. Sambrook, J. et al. (1989) Cold Spring Harbor Laboratory Press, Molecular Cloning: A Laboratory Manual., (2nd ed.), 5.40-5.43.

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