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Product Information Sheet

Product Information	
Klenow Fragment	
Part Number	P7060L 280315
Concentration	5,000 U/mL
Unit Size	2,500 U

Product Specification	
Storage Temperature	-25° to -15°C
TEST:	**SPECIFICATION:**
Purity (SDS-PAGE)	>99%
Specific Activity	5,000 U/mg
SS Exonuclease	Functional
DS Exonuclease	Functional
DS Endonuclease	50 U = no conversion
***E.coli* DNA Contamination**	50 U < 10 copies

Product Description:

Klenow Fragment is a mesophilic DNA polymerase derived from the *E.coli* Polymerase I DNA-dependent repair enzyme. The enzyme exhibits DNA synthesis and proofreading (3'→5') nuclease activities, and, in the absence of the holoenzyme's (5'→3') nuclease domain, displays a moderate strand displacement activity during DNA synthesis. The protein is expressed as a truncated product of the *E.coli* PolA gene.

Source of Protein

A recombinant *E. coli* strain carrying the Klenow Fragment gene.

Supplied in

100 mM KPO_4
1.0 mM DTT
0.1mM EDTA
50% glycerol
pH 7.4 @ 25°C

Supplied with

B0110 (10X Blue Buffer)

10X Blue Buffer (B0110):
500 mM NaCl
100 mM Tris-HCl
100 mM $MgCl_2$
10 mM DTT
pH 7.9 @ 25°C

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 Klenow (3'-5' exo-) storage solution ($[Klenow]_f$ = 0.12-0.002µg/µL) and added to 50 µL reactions containing 4 µg Calf Thymus DNA, 1X Klenow Reaction Buffer, 4mCi/mL ^{3}H-dTTP and 100 µM dNTPs. 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 3.0 µL sample of enzyme 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 product storage solution. The observed average measurement of 3 replicate samples was converted to mg/mL using an extinction coefficient of 55,330 and molecular weight of 68,202 Daltons.

SDS-Page (Physical Purity Assessment) and Specifications

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. Following electrophoresis, the gel was stained and the samples compared to determine physical purity. 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.

Contaminationl Tests:

Single-Stranded Exonuclease Activity

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

Double-Stranded Exonuclease Activity

A 50 µL reaction containing 5,000 cpm of a radiolabeled double-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C resulted in greater than 50% release of TCA-soluble counts.

Double-Stranded Endonuclease Activity

A 50 µL reaction containing 1 µg of pBR322 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 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. The acceptance criterion for the test is the threshold cycle count (C_t) produced by the average of 3 replicate no template control samples. Based on the correlation between the no template control C_t values, and standard curve data, the detection limit of this assay is <10 copies genome/sample.

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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.