



Product Information	
<b>Klenow (3'→5' exo-)</b>	
Part Number:	P701-HC-L
Lot Number:	

Rev.B

### Product Description:

Klenow (3'→5' exo-) is a mesophilic DNA polymerase deficient in both proofreading (3'→5') and nick-translation (5'→3') nuclease activities, and that displays a moderate strand displacement activity during DNA synthesis. The protein is expressed as a truncated product of the *E.coli* PolA gene and contains the D355A and E357A mutations.

### Source of Protein

A recombinant *E. coli* strain carrying the Klenow (3'→5' exo-) gene.

### Supplied in

20 mM Tris-HCl  
1 mM dithiothreitol  
0.1 mM EDTA  
50% glycerol  
pH 7.5 @ 25°C

### Supplied With

B011 (10X Blue Buffer)

### 10X Blue Buffer (B011)

500mM NaCl  
100 mM Tris-HCl  
100 mM MgCl<sub>2</sub>  
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.

### Product Specification\*

Unit Size:	10,000 Units
Unit Concentration	50,000 U/mL
Protein Concentration	5.0 mg/mL
Purity (SDS-PAGE)	>99%
Specific Activity	10,000 U/mg
SS Exonuclease	500 U <0.1% released
DS Exonuclease	500 U <0.1% released
Endonuclease	500 U <0.1% converted
<i>E.coli</i> 16S rDNA Contamination	500 U <10 copies
Storage	-20°C

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

### 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]<sub>f</sub> = 0.12-0.002µg/µL) and added to 50 µL reactions containing 4 µg Calf Thymus DNA, 1X Blue Buffer, 4mCi/mL <sup>3</sup>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*).

#### 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. 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.

#### Protein Concentration (OD<sub>280</sub>) Measurement

A 3.0 µL sample of enzyme was analyzed at OD<sub>280</sub> 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 55,450 and molecular weight of 68,067 Daltons. Acceptance for this assay is +/- 5% of reference sample.

## **Nuclease Contamination Tests:**

### **Single-Stranded Exonuclease Activity**

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

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### **Endonuclease Activity**

A 50  $\mu$ L reaction containing 1  $\mu$ g of pENZuC DNA and 10  $\mu$ L 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  $\mu$ L 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.



#### **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.