Product Information	
Klenow Fragment	
Part Number	P7060L
Concentration	5,000 U/mL
Unit Size	2,500 U
Storage Temperature	-25°C to -15°C
Lot Number	
Reference Number	

Product Description: Klenow Fragment is a mesophilic DNA polymerase derived from the *E. coli* Polymerase I. The enzyme exhibits DNA synthesis and proofreading (3'→5') nuclease activities (1), 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.

Product Specifications						
P7060						
Assay	**SDS Purity**	**Specific Activity**	**SS Exonuclease**	**DS Exonuclease**	**DS Endonuclease**	***E. coli* DNA Contamination**
Units Tested	**n/a**	**n/a**	**50**	**50**	**50**	**50**
Specification	>99%	5,000 U/mg	Functional	Functional	No Conversion	<10 copies

Source of Protein: A recombinant *E. coli* strain carrying a fragment of the PolA gene.

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.

Molecular weight: 68.2 KDa

Quality Control Analysis:

Unit Activity is measured using a 2-fold serial dilution method. Dilutions of enzyme were made in a 50% glycerol Klenow (3'-5' exo-) storage solution and added to 50 µL reactions containing Calf Thymus DNA, 1X Klenow Reaction Buffer, ^{3}H-dTTP and 100 µM dNTPs. Reactions were incubated 10 minutes at 37°C, plunged in ice, and analyzed using the method of Sambrook and Russell (2).

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:
100 mM KPO_4, 1 mM DTT, 0.1 mM EDTA, 50% glycerol (pH 7.5 at 25°C)

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

Usage Instructions: Remove 3' overhangs and fill-in 5' overhangs (3)

1. Set up the following reaction mixture:

- Dissolve DNA in 1X Blue Buffer (B0110)
- Add dNTPs (each at a final concentration of 33 µM)
- Add 1 U of Klenow Fragment (P7060) per microgram of DNA

2. Incubate reaction mixture at 25°C for 15 minutes.
3. Stop the reaction by adding EDTA (final concentration of 10mM) and heating at 75°C for 20 minutes.

References:
1. Jacobsen, H. et al. (1974) Eur. J. Biochem., 45, 623-627.
2. Sambrook, J. and Russell, D.W. (2001) Cold Spring Harbor Laboratory Press, Molecular Cloning: A Laboratory Manual., v3, A8.25-A8.26.
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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