Product Information	
Klenow (3'→5' exo-)	
Part Number	P7010-LC-L
Concentration	5,000 U/mL
Unit Size	10,000 U
Storage Temperature	-25ºC to -15ºC
Lot Number	
Reference Number	

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 (1).

Product Specifications							
P7010							
Assay	**SDS Purity**	**Specific Activity**	**SS Exonuclease**	**DS Exonuclease**	**DS Endonuclease**	***E. coli* DNA Contamination**	**UDG Activity**
Units Tested	**n/a**	**n/a**	**500**	**500**	**500**	**500**	**n/a**
Specification	>99%	10,000 U/mg	<10.0% Released	<1.0% Released	No Conversion	<10 copies	<20 U/mL

Source of Protein: A recombinant *E. coli* strain carrying the Klenow (3'→5' exo-) 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: 68,100 Daltons

Quality Control Analysis:

Unit Activity is measured using a 2-fold serial dilution method. Dilutions of enzyme were made in a glycerol (50%) containing Klenow (3'→5' exo-) storage solution and added to 50 µL reactions containing Calf Thymus DNA, 1X Blue Buffer, ^{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}) 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 **r**eplicate 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.

UDG Contamination is assessed in a 50 µL reaction containing tritiated Uracil DNA and 10 µL enzyme solution incubated for 60 minutes at 37°C under standard UDG unit characterization conditions.

Supplied in: 20mM Tris-HCl, 1mM DTT, 0.1mM EDTA, 50% glycerol (pH 7.5 at 25ºC).
Supplied with:
10X Blue Buffer (B0110): 500mM NaCl, 100mM Tris-HCl, 100mM $MgCl_2$, 10mM DTT (pH 7.9 at 25ºC).

Usage Instructions: Labelling of 3‘ termini with Klenow Fragment (3’-5‘ exo-)

1. Set up the following reaction mixture in a total volume of 50 µl:
   - 1–5 µg purified DNA containing blunt-ends
   - 5 µl 10x Blue Buffer
   - 0.5 µl dATP (10 mM) to a final concentration of 100 µM
   - Add 3 Units Klenow 3’-5’ exo- per microgram DNA
   - Add Nuclease-free water up to 50 µl
2. Incubate at 37°C for 30 minutes.
3. Clean-up the labelled DNA using a spin column-based method (e.g., QIAamp DNA Micro Kit (50), cat. no. 56304)

Reference:

1. Derbyshire, V. et al. (1988). Science. 240, 199-201.

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. SDS sheets relevant to this product are available upon request.