

Klenow (3'–5' exo-)

Catalog #: ENZ-44001-U020

Expiration: One year from date of receipt

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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* Pol A gene and contains the D355A and E357A mutations.

SPECIFICATIONS

Quantity:	20µl
Unit Concentration:	50 U/µl
Protein Concentration:	5.0mg/mL
Appearance:	Clear
Purity:	>99% (SDS-PAGE)
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

Source of Protein

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

Stability and Storage

Store enzyme at -20°C in a non frost-free freezer upon receipt. Minimize the exposure to repeated freeze-thawing by dispensing your stock into experimental quantities. With proper storage, the enzyme is stable for at least one year from date of receipt.

Shipping Conditions

Dry ice (-70°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 ($[Klenow]_f = 0.12-0.002\mu\text{g}/\mu\text{l}$) and added to 50 μl reactions containing 4 μg Calf Thymus DNA, 1X Blue Buffer, 4m Ci/mL ³H-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 (OD₂₈₀) Measurement

A 3.0 μl sample of enzyme was analyzed at OD₂₈₀ using a Nanodrop ND-1000 spectrophotometer standardized using a 2.0mg/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 50µl reaction containing 15,000 cpm of a radiolabeled single-stranded DNA substrate and 10µl of enzyme solution incubated for 4 hours at 37°C resulted in less than 0.1% release of TCA-soluble counts.

Double-Stranded Exonuclease Activity

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

Endonuclease Activity

A 50µl reaction containing 1µg of pENZuC DNA and 10µl 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 5µl 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 (Ct) produced by the average of 3 replicate no template control samples. Based on the correlation between the no template control Ct values, and standard curve data, the detection limit of this assay is <10 copies genome/sample.

ORDERING INFORMATION

Product	Quantity	Cat. No.
Klenow (3'-5' exo-)	20µl	ENZ-44001-U020



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GLOBAL HEADQUARTERS

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