Klenow (3′-5′ exo-)

N-terminal truncated DNA Polymerase I which retains polymerase activity, but no 5´→3´ or 3´→5´exonuclease activity, used for DNA labeling.

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.

Ordering Information

Order Online »

ENZ-44001-U020	20µl

Manuals, SDS & CofA

View Online »

Handling & Storage

Use/Stability	As indicated on product label or CoA when stored as recommended. Stable for at least 1 year after receipt when stored at -20°C.
Handling	Minimize repeated freeze/thaw cycles by aliquotting the stock into experimental quantities.
Long Term Storage	-80°C
Shipping	Dry Ice

Regulatory Status

RUO - Research Use Only

Product Details

Alternative Name	Klenow fragment
Appearance	Clear.
Concentration	Unit Concentration: 50 U/µlProtein Concentration: 5 mg/ml
Formulation	Liquid. In 20mM TRIS-HCl, pH 7.5, containing 50% glycerol, 0.1mM EDTA and 1mM DTT.
Purity	≥99% (SDS-PAGE)

Quality Control

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 µg/µl) and added to 50 µl reactions containing 4 µg Calf Thymus DNA, 1X Blue Buffer, 4m Ci/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).

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_{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 55,450 and molecular weight of 68,067 Daltons. Acceptance for this assay is +/- 5% of reference sample.

Quantity

20µl

Source

Recombinant *E. coli*. carrying the Klenow (3′-5′ exo-) gene

Specific Activity

10,000 U/mg

Specificity

SS Exonuclease: 500U <0.1% released
DS Exonuclease: 500U <0.1% released
Endonuclease: 500U <0.1% converted
E. coli 16S rDNA Contamination 500U <10 copies

UniProt ID

P00582[324-928]

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