

## Klenow DNA Polymerase, exo<sup>-</sup>

Cat.#  
KP002S  
KP002L  
KP002H

Size  
200 units  
1,000 units  
1,000 units

Store at -20°C

Supplied with: 10X Klenow DNA Polymerase Buffer  
Sterile water

### India Contact:

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### Product description

Klenow DNA polymerase, exo<sup>-</sup> is an N-terminal truncation of DNA polymerase I purified from a recombinant source. It has lost the 5' → 3' exonuclease activity but retains polymerase activity. D355A, E357A mutations abolish the 3' → 5' exonuclease activity.

### Characteristics

- Isolated from a recombinant source
- Generates probes using random primers
- Dideoxy sequencing
- Moderate strand displacement activity

### Applications

- Random priming labeling
- DNA sequencing by the Sanger dideoxy method
- Second strand cDNA synthesis
- Second strand synthesis in mutagenesis protocols

### Unit Definition

One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 minutes at 37°C.

### Storage Conditions

25 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, pH 7.4 @ 25°C, Store at -20°C.

*For Research Use Only. Not for use in diagnostic procedures.*

ISO9001 ISO14001 ISO13485

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### Heat Inactivation

75°C for 20 min

### Quality Control

- DNase Activity
- Purity: >99% on SDS-PAGE
- Endonuclease-free
- Exonuclease-free

### Cautions

- Klenow Fragment (exo<sup>-</sup>) remove non-templated 3' additions and not suitable for blunt ends generation.
- Klenow Fragment (exo<sup>-</sup>) is also active in all four EzBuffers in the presence of with dNTPs.
- unit/5 µl reaction volume is recommended for sequencing with Klenow Fragment (exo<sup>-</sup>) by dideoxy method of Sanger et al.

### Standard PCR conditions

- Fill-in reaction of 3' ends of duplex DNA

10X Klenow DNA Polymerase Buffer	2 µl
Klenow DNA Polymerase (5 units/µl)	1 µl
dNTP Mixture (0.5 mM each)	0.5 µl
DNA digested with restriction endonucleases (0.1-4 µg/µl)	1 µl
Sterile water	up to 20 µl

→ Incubate at 37°C for 10 min.

→ Terminate reaction by incubating at 75°C for 20 min.

- Radioisotope labeling of double-strand DNA with recessed 3' ends

10X Klenow DNA Polymerase Buffer	2 µl
Klenow DNA Polymerase (5 units/µl)	0.2 µl
dNTP Mixture (0.5 mM each)	2.5 µl
[α-32P] dNTP (3,000 Ci/mmol)	80 µCi
Digested DNA (0.1-4 µg/µl)	1 µl
Sterile water	up to 20 µl

→ Incubate at 37°C for 10 min.

→ Terminate reaction by incubating at 75°C for 20 min.

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