



## PRODUCT INFORMATION

# Klenow Fragment, $\text{exo}^-$

Pub. No. MAN0012032

Rev. Date 17 January 2017 (Rev. B.00)

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Lot: \_                      Expiry Date: \_

Store at -20 °C

Components	#EP0421	#EP0422
Klenow Fragment, $\text{exo}^-$ , 5 U/ $\mu\text{L}$	300 U	1500 U
10X Reaction Buffer	1 mL	5 × 1 mL

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## Description

Klenow Fragment,  $\text{exo}^-$ , is the Large Fragment of DNA Polymerase I. It exhibits 5'→3' polymerase activity, but lacks the 3'→5' and 5'→3' exonuclease activities of DNA Polymerase I. The 3'→5' exonuclease activity of the enzyme is eliminated by mutations in the 3'→5' exonuclease active site (1).

## Applications

- Random-primed DNA labeling (2-4).
- Labeling by fill-in 5'-overhangs of dsDNA.
- Strand displacement amplification (SDA) (5).
- DNA sequencing by the Sanger method (6).

## Source

*E. coli* cells with a cloned DNA fragment of the mutated *polA* gene.

## Molecular Weight

68 kDa monomer.

## Definition of Activity Unit

One unit of the enzyme catalyzes the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction in 30 min at 37 °C.

## Storage Buffer

The enzyme is supplied in: 25 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM DTT and 50% (v/v) glycerol.

## 10X Reaction Buffer

500 mM Tris-HCl (pH 8.0 at 25 °C), 50 mM MgCl<sub>2</sub>, 10 mM DTT.

## Inhibition and Inactivation

- Inhibitors: metal chelators, PP<sub>i</sub>, P<sub>i</sub> (at high concentrations) (7).
- Inactivated by heating at 75 °C for 10 min or by the addition of EDTA.

## Note

- Klenow Fragment, exo<sup>-</sup>, is not recommended for DNA blunting reactions prior to DNA ligation since it frequently adds one or more extra nucleotides to the 3'-terminus of blunt-end DNA substrates in a non-template directed fashion (8).
- Activity of Klenow Fragment, exo<sup>-</sup>, in Thermo Scientific buffers (in comparison to activity in assay buffer):

Buffers	Activity, %
for restriction enzymes:	
Thermo Scientific™ FastDigest™, FastDigest Green, O, R, 1X Thermo Scientific™ Tango™, 2X Tango, BamHI, EcoRI	100
Ecl136II, PaeI, SacI	50-75
KpnI	75-100
B, G	25-50
for <i>Taq</i> with KCl, <i>Taq</i> with (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> and <i>Pfu</i>	100
for RT buffers	100

## CERTIFICATE OF ANALYSIS

### Endodeoxyribonuclease Assay

No detectable degradation was observed after incubation of supercoiled plasmid DNA with Klenow Fragment, exo<sup>-</sup>.

### Labeled Oligonucleotide (LO) Assay

No detectable degradation after incubation of single-stranded or double-stranded radiolabeled oligonucleotides with Klenow Fragment, exo<sup>-</sup>.

Quality authorized by:



Jurgita Zilinskiene

(continued on back page)

## Protocol for Radioactive Random-primed DNA Labeling

1. Prepare the following reaction mixture:

<b>DNA template</b>	10 $\mu$ L (100 ng)
<b>10X reaction buffer</b>	5 $\mu$ L
<b>12.5 A<sub>260</sub> units/mL (125 <math>\mu</math>M) random decamer primer</b> <i>or</i> <b>7.5 A<sub>260</sub> units/mL (125 <math>\mu</math>M) random hexamer primer</b>	10 $\mu$ L
<b>Water, nuclease-free (#R0581)</b>	to 40 $\mu$ L

2. Incubate the mixture in a boiling water bath for 5-10 minutes and then chill on ice. Spin briefly.

3. Add:

<b>3 dNTP Mix, 0.33 mM each</b> (without a labeled dNTP)	3 $\mu$ L (0.02 mM final concentration)
<b>[<math>\alpha</math>-<sup>32</sup>P]-dNTP, ~110 TBq/mmol</b> (3000 Ci/mmol)	1.85 MBq (50 $\mu$ Ci)
<b>Klenow Fragment, <i>exo</i><sup>-</sup></b>	1 $\mu$ L (5 U)
<b>Water, nuclease-free (#R0581)</b>	to 50 $\mu$ L

4. Incubate the reaction mixture with the random decamer primer at 37 °C for 5 minutes or with the hexamer primer for 10 minutes.

5. Add 4  $\mu$ L 0.25 mM dNTP mix and incubate at 37 °C for 5 minutes.

6. Add 1  $\mu$ L 0.5 M EDTA, pH 8.0 (#R1021) to stop the reaction.

7. Remove 1  $\mu$ L of the reaction mixture and determine the percentage of label incorporated.

8. Purify by using Sephadex G-50 or Bio-Gel P-60.

## Protocol for DNA 3'-end labeling by fill-in of 5'-overhangs

1. Prepare the following reaction mixture:

<b>Linear DNA (aqueous solution)</b>	0.1-4 $\mu$ g
<b>10X reaction buffer</b>	2 $\mu$ L
<b>[<math>\alpha</math>-<sup>32</sup>P]-dNTP, ~15-30TBq/mmol</b> (400-800Ci/mmol)	0.74 MBq (20 $\mu$ Ci)
<i>or</i>	
<b>[<math>\alpha</math>-<sup>32</sup>P]-dNTP, ~110TBq/mmol</b> (3000Ci/mmol)	2.96 MBq (80 $\mu$ Ci)
<b>3 dNTP Mix, 2 mM each</b> (without a labeled dNTP)	2.5 $\mu$ L (0.25 mM final concentration)
<b>Klenow Fragment, <i>exo</i><sup>-</sup></b>	0.2 $\mu$ L (1 U)
<b>Water, nuclease-free (#R0581)</b>	to 20 $\mu$ L

2. Incubate the mixture at 30 °C for 15 minutes.

3. Stop the reaction by heating at 75 °C for 10 minutes.

### Note

The enzyme incorporates modified nucleotides (e.g. Cy3-, Cy5-, fluorescein-, rhodamine-, aminoallyl-, biotin-labeled nucleotides).

## References

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