Klenow Fragment 3'→5' exo$^-$

Catalog: RK20526
Size: 200 U/1,000 U/5,000 U
Concentration: 5,000 U/ml
Components:

Klenow Fragment 3'→5' exo$^-$	RM20516
10X ABuffer B	RM20126

Product Description

Klenow Fragment (3´→ 5´ exo$^-$) is an N-terminal truncation of DNA Polymerase I, which retains polymerase activity, but has lost the 5´→ 3´ exonuclease activity and has mutations (D355A, E357A) which abolish the 3´→ 5´ exonuclease activity.

Klenow Fragment (3´→ 5´ exo$^-$) is isolated from a recombinant source. It generates probes using random primers and shows moderate strand displacement activity.

It can be used in random primer labeling, DNA sequencing by the Sanger dideoxy method, second-strand cDNA synthesis and second-strand synthesis in mutagenesis protocols.

Product Source: An *E. coli* strain containing a plasmid with a fragment of the *E. coli* polA (D355A, E357A) gene starting at codon 324.

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTPs 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
Storage Temperature: -20°C

Reaction Conditions: 1X ABuffer B, Incubate at 37°C
1X ABuffer B: 10 mM Tris-HCl, 50 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT, pH7.9 @ 25°C

Heat Inactivation: 75°C for 20 min
Molecular Weight: Theoretical 68000 daltons
5' - 3' Exonuclease: No
3' - 5' Exonuclease: No
Strand Displacement: +++
Error Rate: ~ 100 x 10^{-6} bases

Instructions

A-Tailing with Klenow Fragment (3'→5' exo$^-$)

Starting Material:
1-5 µg of blunt-ended DNA* (100-1000 bp).
**If starting with blunt-ended DNA that has been prepared by PCR or by end polishing, DNA must be purified to remove the blunting enzymes.*

1. Mix the following components in a sterile microfuge tube:

Composition	Amount
ddH_2O	to 50 µl
10X ABuffer B	5 µl
Purified Blunt DNA	1-5 µg
dATP (10 mM)	0.5 µl (0.1 mM final)
Klenow Fragment (3'→5' exo$^-$)	3 µl (15 U)

2. Incubate in a thermal cycler for 30 minutes at 37°C.

3. Purify DNA sample in one spin column.

Notes:

- Klenow Fragment (3´→ 5´ exo$^-$) is not suitable for generating blunt ends because it lacks the 3´→ 5´ exonuclease necessary to remove non-templated 3´ additions.
- Klenow Fragment (3´→ 5´ exo$^-$) is also active in all ABuffer A/B/C/S when supplemented with dNTPs.
- When Klenow Fragment (3´→ 5´ exo$^-$) is used to sequence DNA using the dideoxy method of Sanger *et al.*, 1 unit/5 µl reaction volume is recommended.

QC Process:

- Purity is above 95% detected by SDS-PAGE.
- No exonuclease, nuclease, RNase contamination.
- No residual host genomic DNA detected by PCR.