Klenow Fragment 3'→5' exo-

Cat. No. : RK20526

Product components

Components	Component number	Concentration	200 U	1,000 U	5,000 U
Klenow Fragment 3'→5' exo-	RM20516	5,000 U/mL	40 μL	200 μL	1 mL
10X ABuffer B	RM20126	10X	1.25 mL	1.25 mL	1.25 mL × 4

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.

Storage

-20℃

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℃.

Reaction Conditions

1X ABuffer B, Incubate at 37℃

1X ABuffer B

10 mM Tris-HCl, 50 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.9 @ 25℃

Storage Conditions

25 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, pH 7.4 @ 25℃

Heat Inactivation

75℃ for 20 min

Molecular Weight

Theoretical 68000 daltons

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Strand Displacement

+++

Error Rate

~ $100x10^{-6}$ 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

1. 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.
2. Klenow Fragment 3′→5′ exo- is also active in all ABuffer A/B/C/S when supplemented with dNTPs.
3. 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.

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