thermo scientific

PRODUCT INFORMATION

Klenow Fragment, exo$^-$

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#_

Lot: _ Expiry Date: _

Store at -20 °C

Components	#EP0421	#EP0422
Klenow Fragment, exo$^-$, 5 U/µL	300 U	1500 U
10X Reaction Buffer	1 mL	5 × 1 mL

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Description

Klenow Fragment, 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 mMTris-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 $MgCl_2$, 10 mM DTT.

Inhibition and Inactivation

- Inhibitors: metal chelators, PP_i, P_i (at high concentrations) (7).
- Inactivated by heating at 75 °C for 10 min or by the addition of EDTA.

Note

- Klenow Fragment, exo⁻, 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⁻, 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
EcI136II, PacI, SacI	50-75
KpnI	75-100
B, G	25-50
for *Taq* with KCl, *Taq* with $(NH_4)_2SO_4$ and *Pfu*	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⁻.

Labeled Oligonucleotide (LO) Assay

No detectable degradation after incubation of single-stranded or double-stranded radiolabeled oligonucleotides with Klenow Fragment, exo⁻.

Quality authorized by:  Jurgita Zilinskiene

(continued on back page)

Protocol for Radioactive Random-primed DNA Labeling

1. Prepare the following reaction mixture:

DNA template	10 µL (100 ng)
10X reaction buffer	5 µL
12.5 A_{260} units/mL (125 µM) random decamer primer *or* **7.5 A_{260} units/mL (125 µM) random hexamer primer**	10 µL
Water, nuclease-free (#R0581)	to 40 µL

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

3 dNTP Mix, 0.33 mM each (without a labeled dNTP)	3 µL (0.02 mM final concentration)
[α-^{32}P]-dNTP, ~110 TBq/mmol (3000 Ci/mmol)	1.85 MBq (50 µCi)
Klenow Fragment, exo$^-$	1 µL (5 U)
Water, nuclease-free (#R0581)	to 50 µL

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 µL 0.25 mM dNTP mix and incubate at 37 °C for 5 minutes.
6. Add 1 µL 0.5 M EDTA, pH 8.0 (#R1021) to stop the reaction.
7. Remove 1 µL 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:

Linear DNA (aqueous solution)		0.1-4 µg
10X reaction buffer		2 µL
[α-^{32}P]-dNTP,	~15-30TBq/mmol (400-800Ci/mmol)	0.74 MBq (20 µCi)
or		
[α-^{32}P]-dNTP,	~110TBq/mmol (3000Ci/mmol)	2.96 MBq (80 µCi)
3 dNTP Mix, 2 mM each (without a labeled dNTP)		2.5 µL (0.25 mM final concentration)
Klenow Fragment, exo$^-$		0.2 µL (1 U)
Water, nuclease-free (#R0581)		to 20 µL

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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