thermo scientific

PRODUCT INFORMATION

Klenow Fragment

Pub. No. MAN0012882
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Lot: _ Expiry Date: _

Store at -20 °C

Components	#EP0051	#EP0052	#EP0054
Klenow Fragment	300 U 10 U/µL	1500 U 10 U/µL	300 U 2 U/µL
10X Reaction Buffer	1 mL	5 × 1 mL	1 mL

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Description

Klenow Fragment is the Large Fragment of DNA Polymerase I, *E.coli*. It exhibits 5'→3' polymerase activity and 3'→5' exonuclease (proofreading) activity, but lacks 5'→3' exonuclease activity of DNA Polymerase I.

Applications

- DNA blunting by fill-in of 5'-overhangs or removal of 3'-overhangs. (1), *see* protocols on back page.
- Random-primed DNA labeling (2-4).
- Labeling by fill-in 5'-overhangs of dsDNA.
- DNA sequencing by the Sanger method (5).
- Site-specific mutagenesis of DNA with synthetic oligonucleotides (6).
- Second strand synthesis of cDNA (7).

Source

E.coli cells with a cloned fragment of the *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 $MgCl_2$, 10 mM DTT.

Inhibition and Inactivation

- Inhibitors: metal chelators, PP_i, P_i (at high concentrations) (8).
- Inactivated by heating at 75 °C for 10 min or by addition of EDTA.

Note

- Activity of Klenow Fragment 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, PacI, SacI, KpnI	50-75
B	25-50
G	20-50
for PCR buffers: *Taq* buffer with KCl, *Taq* buffer with $(NH_4)_2SO_4$, *Pfu* buffer	100
RT buffers	100

CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay

No detectable degradation was observed after incubation of supercoiled plasmid DNA with Klenow Fragment.

Quality authorized by: Jurgita Zilinskiene

(continued on back page)

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

1. Prepare the following reaction mixture:

Linear DNA	0.1-4 µg
10x reaction buffer for Klenow Fragment	2 µL
[α-^{32}P]-dNTP, **~15-30 TBq/mmol (400-800 Ci/mmol)** ***or*** **[α-^{32}P]-dNTP,** ~110 TBq/mmol (3000 Ci/mmol)	0.74 MBq (20 µCi) 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	0.1 µL (1 U)
Water, nuclease-free (#R0581)	to 20 µL
Total volume	20 µL

2. Incubate at 37 °C for 15 min.
3. Stop the reaction by heating at 75 °C for 10 min.

Note

This protocol is suitable for labeling of the following DNA markers, composed of DNA fragments with 5'-overhangs:
Lambda DNA EcoRI Marker, #SM0281
Lambda DNA HindIII Marker, #SM0101
Lambda DNA EcoRI/HindIII Marker, #SM0191

- The modified version of this protocol can be used for nonradioactive labeling of DNA markers. Substitute a part of dTTP with a modified nucleotide (e.g. Biotin-11-dUTP or Fluorescein-12-dUTP) at a molar ratio of 1:2.

Protocol for DNA Blunting by fill-in of 5'-overhangs or removal of 3'-overhangs

1. Prepare the following reaction mixture:

Linear DNA	10-15 µL (0.1-4 µg)
10X reaction buffer for Klenow Fragment	2 µL
dNTP Mix, 2mM each (#R0241)	0.5 µL (0.05 mM final concentration)
Klenow Fragment	0.1-0.5 µL (1-5 U)
Water, nuclease-free (#R0581)	to 20 µL
Total volume	20 µL

2. Mix thoroughly, spin briefly and incubate at 37 °C for 10 min.
3. Stop the reaction by heating at 75 °C for 10 min.

Note

The enzyme incorporates modified nucleotides (e.g. biotin-, digoxigenin-, fluorescently-labeled nucleotides).

References

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