Thermo SCIENTIFIC

PRODUCT INFORMATION

Klenow Fragment

#EP0051 300 u

Lot: **Expiry Date:**

Concentration: 10 u/µl
Supplied with: 1 ml of 10X Reaction Buffer

Store at -20°C

In total 2 vials.

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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. (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 (adsorbed on DE-81) in 30 min at 37°C.
Enzyme activity is assayed in the following mixture: 50 mM Tris-HCl (pH 8.0 at 25°C), 5 mM $MgCl_2$, 1 mM DTT, 0.033 mM dNTP, 0.4 M Bq/ml [^{3}H]-dTTP and 62.5 µg/ml activated salmon milt DNA.

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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
EcI136II, 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 conversion of covalently closed circular DNA to nicked DNA was detected after incubation of 20 units of Klenow Fragment with 1 µg of pUC19 DNA for 4 hours at 37°C.

Quality authorized by: Jurgita Zilinskiene

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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 Fermentas 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
Lambda DNA Eco91I Marker, #SM0111
ΦX174 DNA HinfI Marker, #SM0261

- 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 Fill-in of 5'-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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