

CERTIFICATE OF ANALYSIS

Klenow Fragment

#EP0054 LC, 300 u

Lot: **Expiry Date:**

Concentration: 2 u/μl
Supplied with: 1 ml of 10X Reaction Buffer

Store at -20°C

In total 2 vials.

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.

Source

E.coli cells with a cloned fragment of the *polA* gene.

Molecular Weight

68kDa 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 30min at 37°C.

Enzyme activity is assayed in the following mixture: 67mM potassium phosphate (pH 7.4), 6.7mM MgCl₂, 1mM 2-mercaptoethanol, 0.033mM dATP, 0.033mM dTTP, 0.4MBq/ml [³H]-dTTP and 62.5μg/ml poly(dA-dT)·poly(dA-dT).

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 MgCl₂, 10 mM DTT.

Applications

- Filling-in or labeling recessed 3'-termini of double-stranded DNA (1), see protocols on back page.
- Random-primed DNA labeling (2-4).
- DNA sequencing by the Sanger method (5).
- Site-specific mutagenesis of DNA with synthetic oligonucleotides (6).
- Second strand synthesis of cDNA (7).

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 Fermentas buffers (in comparison to activity in assay buffer):

Buffers	Activity, %
for restriction enzymes: O, R, 1X Tango™, 2X Tango™, BamHI, EcoRI Ecl136II, SacI, KpnI B, G	100 50-75 25-50
for <i>Taq</i> and <i>Pfu</i> DNA polymerases	100
for M-MuLV reverse transcriptases	100


QUALITY CONTROL ASSAY DATA

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 20 units of Klenow Fragment with 1 µg of pBR322 DNA in 50 µl of reaction buffer for 4 hours at 37°C.

Functional Assay

Klenow Fragment was tested for filling-in recessed 3'-termini of DNA.

Quality authorized by:  Jurgita Zilinskiene

(continued on back page)

Protocol for Labeling Recessed 3'-termini of Double-stranded DNA

① Prepare the following reaction mixture:

Digested DNA (aqueous solution)	10-15 μ l (0.1-4 μ g)
10X reaction buffer for Klenow Fragment	2 μ l
$[\alpha\text{-}^{32}\text{P}]\text{-dNTP}$, ~15-30 TBq/mmol (400-800 Ci/mmol)	0.74 MBq (20 μ Ci)
<i>or</i>	
$[\alpha\text{-}^{32}\text{P}]\text{-dNTP}$, ~110 TBq/mmol (3000 Ci/mmol)	2.96 MBq (80 μ Ci)
3 dNTP Mix, 2mM each (without a labeled dNTP)	2.5 μ l (0.25 mM final concentration)
Klenow Fragment	0.1 μ l (1 u)
Water, nuclease-free (#R0581)	to 20 μ l

② Incubate the mixture at 30°C for 15 minutes.

③ Stop the reaction by heating at 75°C for 10 minutes.

Protocol for Fill-in Recessed 3'-termini of Double-stranded DNA

① Prepare the following reaction mixture:

Digested DNA (aqueous solution)	10-15 μ l (0.1-4 μ g)
10X reaction buffer for Klenow Fragment	2 μ l
dNTP Mix, 2mM each (#R0241)	0.5 μ l (0.05 mM final concentration)
Klenow Fragment	0.1-0.5 μ l (1-5 u)
Water, nuclease-free (#R0581)	to 20 μ l

② Incubate the mixture at 37°C for 10 minutes.

③ Stop the reaction by heating at 75°C for 10 minutes.

Note

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

References

1. Ausubel, F.M., et al., Current Protocols in Molecular Biology, vol. 1, John Wiley & Sons, Inc., Brooklyn, New York, 3.5.7-3.5.10, 1994-2005.
2. Feinberg, A.P., Vogelstein, B., A technique for radiolabeling DNA restriction endonucleases fragments to high specific activity, *Anal. Biochem.*, 132, 6-13, 1983.
3. Feinberg, A.P., Vogelstein, B., Addendum to: A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity, *Anal. Biochem.*, 137, 266-267, 1984.
4. Yu, H., et al., Cyanine dye dUTP analogs for enzymatic labeling of DNA probes, *Nucleic Acids Res.*, 22, 3226-3232, 1994.
5. Sanger, F., et al., DNA sequencing with chain-terminating inhibitors, *Proc. Natl. Acad. Sci. USA*, 74, 5463-5467, 1977.
6. Wallace, R.B., et al., Directed deletion of a yeast transfer RNA intervening sequence, *Science*, 209, 1396-1400, 1980.
7. Rougeon, F., et al., Insertion of rabbit β -globin gene sequence into an *E.coli* plasmid, *Nucleic Acids Res.*, 2, 2365-2378, 1975.
8. Eun, H-M., *Enzymology Primer for Recombinant DNA Technology*, Academic Press, Inc., 1996.

Related Products

- dNTP Mix, 2 mM each #R0241
- dNTP Mix, 10 mM each #R0191
- dNTP Mix, 25 mM each #R1121
- dNTP Set #R0181
- Aminoallyl-dUTP #R0091
- Biotin-11-dUTP (not available in the USA) #R0081
- Fluorescein-12-dUTP (not available in the USA) #R0101
- M-MuLV Reverse Transcriptase #EP0351
- RevertAid™ M-MuLV Reverse Transcriptase (not available in the USA) #EP0441
- RevertAid™ H Minus M-MuLV Reverse Transcriptase (not available in the USA) #EP0451
- Ribonuclease H, *E.coli* #EN0201
- Pyrophosphatase, Inorganic (from yeast) #EF0221
- 0.5 M EDTA, pH 8.0 #R1021
- Water, nuclease-free #R0581

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to www.fermentas.com for Material Safety Data Sheet of the product.