Certificate of Analysis

DNA Polymerase I Large (Klenow) Fragment:

Part No.	Size (units)
M220A	150
M220C	500

Part# 9PIM220

Revised 4/18

Enzyme Storage Buffer: Klenow Fragment is supplied in 50mM Tris-HCl (pH 7.5), 1mM DTT, 0.1mM EDTA and 50% (v/v) glycerol.

Klenow 10X Buffer (M195A): The Klenow 10X Buffer supplied with this enzyme has a composition of 500mM Tris-HCl (pH 7.2), 100mM $MgSO_4$ and 1mM DTT.

Source: Recombinant strain of *E. coli* (1).

Storage Conditions: See the Product Information Label for storage recommendations. Avoid exposure to frequent temperature changes. See the expiration date on the Product Information Label.

Unit Definition: One unit is defined as the amount of enzyme required to catalyze the incorporation of 10nmol of deoxynucleotide into acid-precipitable material in 30 minutes at 37°C. The reaction conditions are: 67mM potassium phosphate (pH 7.5), 6.7mM $MgCl_2$, 1mM DTT, 50µg/ml activated calf thymus DNA, and 33µM each of dCTP, dATP, dGTP and [^{3}H]dTTP. See the unit concentration on the Product Information Label.

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Quality Control Assays

Activity Assay

Unit Activity Assay: See unit definition.

Contaminant Activity

Endonuclease Assay: To test for endonuclease activity, 1µg of Form I (supercoiled) plasmid DNA is incubated with 10 units of Klenow Fragment in Klenow 1X Buffer for 5 hours at 37°C. Following incubation, the supercoiled DNA is visualized on an ethidium bromide-stained agarose gel to verify the absence of visible nicking or cutting.

Physical Purity: The purity is >80% as judged by SDS-polyacrylamide gels with Coomassie® blue staining. Klenow Fragment is free from intact DNA polymerase and small fragment as indicated by SDS-polyacrylamide gel electrophoresis.

Reference

1. Joyce, C.M. and Grindley, N.D. (1983) Construction of a plasmid that overproduces the large proteolytic fragment (Klenow fragment) of DNA polymerase I of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **80**, 1830–4.

Signed by:

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Usage Information

I. Description

DNA Polymerase I Large (Klenow) Fragment consists of a single polypeptide chain (68kDa) that lacks the 5´ →3´ exonuclease activity of intact *E. coli* DNA polymerase I but retains its 5´ →3´ polymerase, 3´→5´ exonuclease and strand displacement activities. The 5´ →3´ polymerase activity of Klenow Fragment can be used in the following applications: a) to fill in 5´ -protruding ends with unlabeled or labeled dNTPs (1); b) to sequence single- or double-stranded DNA templates (2); c) for in vitro mutagenesis experiments using synthetic oligonucleotides (3); d) for cDNA second strand synthesis (4); e) to generate single-stranded DNA probes (5). The 3´ →5´ exonuclease activity can be used to generate blunt ends from a 3´ -overhang (6). Klenow Fragment, Exonuclease Minus, which is deficient in both the 5´ →3´ and the 3´ →5´ exonuclease activities, is primarily used for sequencing of DNA templates. For a detailed review of Klenow and other DNA polymerases, see reference 7.

II. Reaction Conditions for Klenow Applications

Many of the applications in which Klenow Fragment is used require restriction digestion of the DNA. We recommend purifying the DNA by using the Wizard® DNA Clean-Up System (Cat.# A7280) or phenol:chloroform extraction and ethanol precipitation before filling 5´ -protruding ends using Klenow Fragment. For optimal activity, use the Klenow 10X Buffer supplied with the enzyme. Klenow Fragment is also active in many restriction enzyme buffers, and some users may choose to perform the fill-in reaction directly in the restriction buffer. For other applications, such as sequencing, the optimal reaction conditions are described in the individual protocols below.

A. Filling 5´ -Protruding Ends with Unlabeled dNTP

Digest 1–4µg of DNA (in a volume of 20µl) with an appropriate restriction enzyme that will generate a 5´-overhang. The optimal reaction conditions for filling are: 50mM Tris-HCl (pH 7.2), 10mM $MgSO_4$, 0.1mM DTT, 40µM of each dNTP, 20µg/ml acetylated BSA and 1 unit of Klenow Fragment per microgram of DNA. Incubate the reaction at room temperature for 10 minutes. Stop the reaction by heating the mixture for 10 minutes at 75°C.

Note: Klenow Fragment, Exonuclease Minus, will leave a single-base 3´-overhang for a significant proportion of the DNA fragments during the fill-in reaction (8). Therefore, these fragments should not be used in blunt end cloning experiments.

B. Filling 5´ Protruding Ends With Labeled dNTP

Digest 1–4µg of DNA (in a volume of 20µl) with an appropriate restriction enzyme that will generate a 5´-overhang. Add 20µCi of the desired [α-^{32}P]dNTP (400–800Ci/mmol), 1µl of an appropriate 5mM dNTP solution and 1–4 units of Klenow Fragment to the reaction mixture. Incubate the reaction for 15 minutes at 30°C. Stop the reaction by adding 1µl of 0.5M EDTA (20mM final concentration) to the mixture, or by heating the mixture for 10 minutes at 75°C. This method is particularly suitable for labeling restriction fragments to use as size standards, since all fragments are labeled equally and will have the same intensity on an autoradiogram (6).

C. Generation of Single-Stranded DNA Probes

Denature the DNA template by heating it in a microcentrifuge tube for 10 minutes at 95–100°C. Rapidly chill the tube in an ice bath. To generate a single-stranded DNA probe, prepare the following reaction mixture: 500ng/ml denatured DNA template (25ng optimum), 50mM Tris-HCl (pH 8.0), 5mM $MgCl_2$, 2mM DTT, 0.2M HEPES (pH 6.6), 150µg/ml random hexadeoxyribonucleotides, 400µg/ml BSA, 20µM of each unlabeled dNTP, 333nM [α-^{32}P]dNTP (3,000Ci/mmol) and 5 units of Klenow Fragment. Incubate the reaction for 60 minutes at room temperature. Stop the reaction by adding 1µl of 0.5M EDTA (20mM final concentration) to the mixture or by heating the mixture for 10 minutes at 75°C.

D. Dideoxy Sequencing

Note: We recommend the use of Klenow Fragment, Exonuclease Minus, for this protocol.

The protocol detailed here is suitable for sequencing both single-stranded and denatured double-stranded DNA templates. If the template is single-stranded, proceed directly to the sequencing protocol. If the template is double-stranded, follow the denaturation procedure outlined below.

Denaturation protocol: Pipet 0.8–4µg of supercoiled plasmid into a microcentrifuge tube and add sterile deionized water to a final volume of 18µl. Add 2µl of 2M NaOH/2mM EDTA and mix the solutions by pipeting. Incubate the DNA for 15 minutes at 37°C. Add 2µl of 2M ammonium acetate (pH 4.6), and vortex to mix. Add 112µl of 95% ethanol, vortex briefly and incubate the tube for 15 minutes at –70°C. Centrifuge the tube for 15 minutes at 12,000 x *g* in a microcentrifuge. Carefully remove the supernatant and wash the pellet with 500µl of cold (–20°C) 70% ethanol. Centrifuge the tube for 5 minutes at 12,000 x *g* in a microcentrifuge. Carefully remove **all** of the supernatant and resuspend the DNA in 5µl of deionized water. Proceed to the sequencing protocol.

Sequencing protocol: In a 0.5ml microcentrifuge tube, combine 0.8–4µg of single-stranded denatured DNA template, 0.8–2pmol of primer (maintain a 1:1 molar ratio of template:primer), 1.5µl of sequencing reaction 10X buffer [70mM Tris-HCl (pH 7.5), 70mM $MgCl_2$, 300mM NaCl, 100mM DTT, 1mM EDTA (pH 8.0)] and sterile, redistilled water to a final volume of 10µl. To anneal the primer to the template DNA, incubate the mixture for 10 minutes at 55°C and then slowly cool it to room temperature. Add 2µl of [α-^{35}S]dATP (1,000Ci/mmol) and 2 units of Klenow Fragment, Exonuclease Minus. Mix the components by pipeting. Centrifuge the tube at 12,000 x *g* for 10 seconds in a microcentrifuge to collect the mixture in the bottom of the tube. Transfer 2.5µl of the reaction mixture to each of 4 tubes marked G, A, T and C. Add 2µl of the appropriate d/ddNTP mix (see Table 1) to each tube, mix by pipeting and centrifuge briefly in a microcentrifuge. Incubate the reaction at room temperature for 20 minutes. Add 2µl of chase solution (125µM of each dNTP) and incubate the reaction for an additional 20 minutes at room temperature. Add 4µl of stop solution (95% formamide, 0.5% xylene cyanol, 0.5% bromophenol blue, 10mM NaOH) to each of the reactions. Heat the reactions for 3 minutes at 95°C just prior to loading them onto a sequencing gel.

Table 1. Composition of dNTP/ddNTP Mixtures.

Nucleotide	G Mixture	A Mixture	T Mixture	C Mixture
7-deaza-dGTP	5µM	100µM	100µM	100µM
dTTP	100µM	100µM	5µM	100µM
dCTP	100µM	100µM	100µM	10µM
ddGTP	120µM	–	–	–
ddATP	–	100µM	–	–
ddTTP	–	–	500µM	–
ddCTP	–	–	–	100µM

III. Additional Information

Molecular Weight: Klenow Fragment is a 68kDa monomer (7).
Heat Inactivation: Klenow Fragment may be inactivated by incubation for 10 minutes at 75°C.
Inhibitors: Klenow Fragment is inhibited by the adenosine analog adenosine 2 ,2 -riboepoxide 5 -triphosphate (9).

IV. References

1. Anderson, S. *et al.* (1980) A short primer for sequencing DNA cloned in the single-stranded phage vector M13mp2. *Nucl. Acids Res.* **8**, 1731–43.
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