

Manual

DNA Polymerase I, Large (Klenow) Fragment

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1. Introduction

DNA Polymerase I, Large (Klenow) Fragment is a DNA-dependent DNA polymerase that lacks the 5'→3' exonuclease activities of *E. coli* DNA Polymerase I from which it is derived. The 3'→5' remains intact to remove 3' overhangs, and along with the polymerase activity to fill in 5' overhangs and create blunt end DNA fragments. The optimal incubation temperature of the enzyme is 25 °C and uses 10X DNA Polymerase Buffer A. Heat inactivation of the enzyme is 75 °C for 20 minutes.

2. Product designations and kit components

Product	Kit size	Catalogue number	Reagent description	Part number	Volume
DNA Polymerase I, Large (Klenow) Fragment Kit	1,000 units	30098-1	DNA Polymerase I, Large (Klenow) Fragment, (5 U/μL)	F836665-1	200 μL
			10X DNA Polymerase Buffer A	F98628-1	1.2 mL

3. Product specifications

Storage: Store at -20 °C in a freezer without a defrost cycle.

Storage buffer: DNA Polymerase I, Large (Klenow) Fragment is supplied in a 50% glycerol solution containing 25 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT.

10X DNA Polymerase Buffer A: 100 mM Tris-HCl (pH 7.9), 100 mM MgCl₂, 10 mM DTT, 500 mM NaCl.

Unit definition: One unit of DNA Polymerase I, Large (Klenow) converts 10 nmol of dNTPs into acid-insoluble material in 30 minutes at 37 °C under standard assay conditions.

Contaminating activity assays: DNA Polymerase I, Large (Klenow) Fragment is free of detectable endonuclease and RNase activities.

4. General protocol

4.1. Prepare reaction set up as follows:

Component	Amount
DNA	Up to 5 μg
dNTPs	50 μM each
10X DNA Polymerase Buffer A	2 μL
DNA Polymerase I, Large (Klenow) Fragment	1 μL (5 units)
Water	Up to 20 μL

4.2. Mix reaction well and briefly spin down.

4.3. Incubate at 25 °C for 30 minutes.

4.4. Heat inactivate the polymerase by incubating at 75 °C for 20 minutes.

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5. References

1. Jacobsen, H., Klenow, H. and Overgaard-Hansen, K. (1974). Eur. J. Biochem.. **45**, 623-627
2. Bebenek, K., Joyce, C.M., Fitzgerald, M.P. and Kunkel, T.A. (1990). J. Bio. Chem . **265**, 13878-13887

6. Further support

If you require any further support, please do not hesitate to contact our Technical and Applications Support Team: techsupport@lgcgroup.com.



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