

Product Contents

DNA Polymerase I:

Part No.	Size (units)
M205A	500
M205B	2,500

DNA Polymerase I 10X Reaction Buffer (M195A): The DNA Polymerase 10X Reaction Buffer supplied with this enzyme has a composition of 500mM Tris-HCl (pH 7.2 at 25°C), 100mM MgSO₄ and 1mM DTT.

Enzyme Storage Buffer: DNA Polymerase I is supplied in 50mM Tris-HCl (pH 7.5 at 25°C), 1mM DTT, 0.1mM EDTA and 50% (v/v) glycerol.

Source: Purified from an *E. coli* strain expressing a recombinant clone (1).

Storage Temperature: See the Product Information Label for storage recommendations. Avoid multiple freeze-thaw cycles and 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 deoxyribonucleotides into trichloroacetic acid insoluble form in 30 minutes at 37°C in 67mM potassium phosphate (pH 7.4), 6.7mM MgCl₂, 1mM DTT, 50µg/ml activated calf thymus DNA and 33µM each dNTP. See the unit concentration on the Product Information Label.

Quality Control Assays

Activity Assay

Unit Activity Assay: See unit definition above.

Contaminant Activity

Endonuclease Assay: To test for endonuclease activity, 1µg of Type I supercoiled plasmid DNA is incubated in 25 units of DNA Polymerase I 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 ≥90% as judged by SDS-polyacrylamide gels with Coomassie® blue staining.

Reference

1. Kelley, W.S., Chalmers, K. and Murray, N.E. (1977) Isolation and characterization of a lambdaDpolA transducing phage. *Proc. Natl. Acad. Sci. USA* **74**, 5632-6.

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I. Description

DNA Polymerase I catalyzes template-directed polymerization of nucleotides into duplex DNA in a 5'→3' direction. DNA Polymerase I also possesses a 3'→5' exonuclease activity or "proofreading" function, which lowers the error rate during DNA replication, and contains a 5'→3' exonuclease activity, which enables the enzyme to replace nucleotides in the growing strand of DNA by nick translation (1). DNA Polymerase I is capable of catalyzing *de novo* synthesis of synthetic homopolymers and provides a convenient method for the preparation of a variety of defined DNA substrates in the laboratory (2).

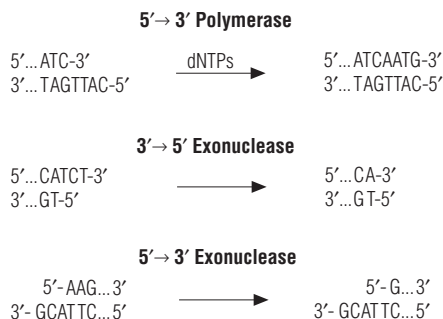


Figure 1. Properties of DNA Polymerase I.

II. Reaction Conditions

A. Filling-In of 5'-Overhang

This procedure has been adapted from reference 3.

Mix the following in a microcentrifuge tube: 8.5μl water, 5μl DNA (1–5μg digested DNA containing 5'-overhangs), 1.5μl 1M Tris-HCl (pH 7.5), 2.5μl 0.1M MgCl₂, 2.5μl 0.1M β-mercaptoethanol, 5μl 1mM dNTP mixture (2μl each of 10mM dATP, dCTP, dGTP, dTTP and 12μl water) and 0.5μl DNA Polymerase I (2.5 units). Incubate at 12°C for 1 hour. Heat at 68°C for 10 minutes to inactivate the enzyme.

B. Blunt-Ending of 3'-Overhang

This procedure has been adapted from reference 3.

The 3'-overhang is first removed by the exonuclease activity of DNA Polymerase I. Because removal of more nucleotides may occur (generating a 5'-protruding end), repair is performed after addition of dNTPs in order to generate a blunt end. In a microcentrifuge tube, prepare a 1mM mixture of the four dNTPs (2μl each of 10mM dATP, dCTP, dGTP, dTTP and 12μl water). In another microcentrifuge tube, mix 8.5μl water, 5μl DNA (containing 1–5μg digested DNA with 3'-overhangs), 1.5μl 1M Tris-HCl (pH 7.5), 2.5μl 0.1M MgCl₂, 2.5μl 0.1M β-mercaptoethanol, 0.5μl DNA Polymerase I (2.5 units). Incubate at 12°C for 10 minutes. Add 5μl of the 1mM dNTP mixture to the DNA. Incubate at 12°C for 1 hour. Heat at 68°C for 10 minutes to inactivate the enzyme.

C. Nick Translation

This reaction may be scaled between 10–100μl volume, but the components should be kept in the same proportions as in the standard reaction. Set up the following reaction in a microcentrifuge tube: 18μl water, 10μl nucleotide mix (prepared by mixing equal volumes of the 3 unlabeled 300μM nucleotides chosen minus the nucleotide selected as label), 5μl nick translation 10X buffer (see Section IV), 5μl sample DNA (at 0.2μg/μl), 7μl [α -³²P]dCTP (400Ci/mmol at 10mCi/ml) and 5μl DNA Polymerase I/DNase I mix (see Section IV). Incubate at 15°C for 1 hour. Add 5μl stop solution (0.25M EDTA [pH 8.0]).

III. Miscellaneous Information

Source: DNA Polymerase I is purified from the recombinant *E. coli* strain CM5199 (4), which is a lysogen carrying a lambda pol A transducing phage (5).

Molecular Weight: 109,000 Daltons.

Activator: Mg²⁺.

Inactivator: 68°C for 10 minutes (3).

IV. Composition of Buffers and Solutions

Nick translation 10X buffer

500mM	Tris-HCl (pH 7.2)
100mM	MgSO ₄
1.0mM	DTT

DNA Polymerase I/DNase I mix

50%	glycerol
50mM	Tris-HCl (pH 7.2)
10mM	MgSO ₄
0.1mM	DTT
0.5mg/ml	nuclease-free BSA
1,000u/ml	DNA Polymerase I
3u/ml	RQ1 RNase-Free DNase (DNase I) (Cat.# M6101)

Prepare the buffer solution and then add the DNA Polymerase I and RQ1 RNase-Free DNase to a final concentration of 1,000u/ml and 3u/ml, respectively.

V. References

- Kelly, R.B. *et al.* (1970) Enzymatic synthesis of deoxyribonucleic acid. XXXII. Replication of duplex deoxyribonucleic acid by polymerase at a single strand break. *J. Biol. Chem.* **245**, 39–45.
- Harwood, S.J. *et al.* (1970) *Micrococcus luteus* deoxyribonucleic acid polymerase. Studies of the enzymic reaction and properties of the deoxyribonucleic acid product. *J. Biol. Chem.* **245**, 5614–24.
- Perbal, B. (1988) *A Practical Guide to Molecular Cloning*, 2nd ed., John Wiley and Sons.
- Kelley, W.S. and Stump, K.H. (1979) A rapid procedure for isolation of large quantities of *Escherichia coli* DNA polymerase I utilizing a lambda polA transducing phage. *J. Biol. Chem.* **254**, 3206–10.
- Kelley, W.S., Chalmers, K. and Murray, N.E. (1977) Isolation and characterization of a lambda polA transducing phage. *Proc. Natl. Acad. Sci. USA* **74**, 5632–6.