Certificate of Analysis

Part# 9PIM205

Revised 9/13

DNAPolymerase 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 $MgSO_4$ 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 $MgCl_2$, 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 lambdapolA transducing phage. *Proc. Natl. Acad. Sci. USA* **74**, 5632–6.

Signed by: J. Stevens

J. Stevens, Quality Assurance

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Usage Information

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).

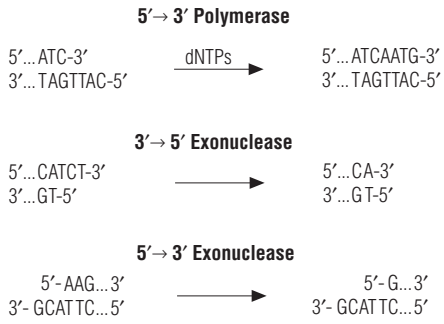

Figure 1. Properties of DNA Polymerase I.

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 $MgCl_2$, 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 $MgCl_2$, 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-^{32}P]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: Mg^{2+}.
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

1. 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.
2. 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.
3. Perbal, B. (1988) *A Practical Guide to Molecular Cloning, 2nd ed.*, John Wiley and Sons.
4. Kelley, W.S. and Stump, K.H. (1979) A rapid procedure for isolation of large quantities of *Escherichia coli* DNA polymerase I utilizing a lambdapolA transducing phage. *J. Biol. Chem.* **254**, 3206–10.
5. Kelley, W.S., Chalmers, K. and Murray, N.E. (1977) Isolation and characterization of a lambdapolA transducing phage. *Proc. Natl. Acad. Sci. USA* **74**, 5632–6.

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