For research use only

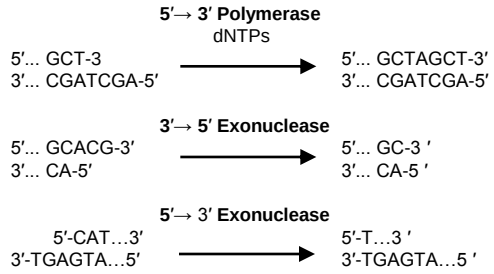
ISO9001

DNA Polymerase I (*E.coli*)

Product	Quantity	Cat. No.	Remarks
DNA Polymerase I (*E.coli*)	200 unit	EBT-1022	5 unit/㎕

Description

DNA Polymerase I from *E.coli* catalyzes the template-directed polymerization of nucleotides into duplex DNA in a 5′→3′ direction. DNA Polymerase I possesses a 3′→5′ exonuclease activity or "proof-reading" function, which lowers the error rate during DNA replication, and also contains a 5′→3′ exonuclease activity, which enables the enzyme to replace nucleotides in the growing strand of DNA by nick translation. The enzyme, purified from recombinant *E.coli*, 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.



Concentration & Storage Condition

5 unit/ ㎕. Store at -20℃.

Storage Buffer

50 mM Tris-HCl, pH 7.5, 1 mM DTT, 0.1 mM EDTA, 50% (v/v) glycerol.

10x Reaction Buffer

500 mM Tris-HCl, pH 7.2, 100 mM $MgSO_4$, 1 mM DTT.

Unit Definition

One unit is defined as the amount of enzyme that incorporates 10 nmole of dNTP into TCA-insoluble material in 30 min at 37°C. The reaction conditions are: 67mM potassium phosphate , pH 7.5, 6.7 mM $MgCl_2$, 1 mM DTT, 50 μg/ml activated calf thymus DNA and 33 mM dATP, dCTP, dGTP and dTTP (a mix of unlabeled and [^{3}H]dTTP).

QC Tests

Activity, exo and endonuclease activity test, SDS-PAGE purity, performance tests.

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Usage Information

1. Fill-In of 5′-Overhang to form Blunt ends

1) Add the following components to the microcentrifuge tube :

DNA (1–5μg digested DNA containing 5′-overhangs)	xμl
10x Reaction Buffer	2μl
1mM dNTP mix (0.25mM each)	5μl
DNA Polymerase I (2.5 units)	0.5μl
Nuclease-Free Water to final volume	20μl

2) Incubate at 37°C for 1 hour.
3) Heat at 75°C for 10 minutes to inactivate the enzyme.

2. Removal of 3′-Overhang to form Blunt ends

The 3′-overhang is first removed by the exonuclease activity of DNA Polymerase I.

1) Add the following components to the microcentrifuge tube :

DNA (1–5μg digested DNA containing 5′-overhangs)	xμl
10x Reaction Buffer	2μl
DNA Polymerase I (2.5 units)	0.5μl
Nuclease-Free Water to final volume	20μl

2) Incubate at 37°C for 10 min.
3) Add 5μl of the 1mM dNTP mixture to the DNA.
4) Incubate at 12°C for 1 hour.
5) Heat at 75°C for 10 min to inactivate the enzyme.

3. Nick Translation

This reaction may be scaled between 10-100μl volume.
Nucleotide mix prepared by mixing equal volumes of the 3 unlabeled 300μM nucleotides chosen minus the nucleotide selected as label.

1) Set up the following reaction in a microcentrifuge tube :

Nucleotide mix	10μl
Nick translation 10X buffer	5μl
Sample DNA (at 0.2μg/μl)	5μl
[α-^{32}P]dCTP (400Ci/mmol at 10mCi/ml)	7μl
DNA Polymerase I/DNase I mix.	5μl
Nuclease-Free Water to final volume	50μl

2) Incubate at 37°C for 1 hour.
3) Add 5μl stop solution (0.25M EDTA (pH 8.0)).

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 RNase-Free DNase (DNase I)

Prepare the buffer solution and then add the DNA Polymerase I and RNase-Free DNase.