For research use only

ISO9001

DNA Polymerase I Large (Klenow) fragment

Product	Quantity	Cat. No.	Remarks
DNA Polymerase I Large (Klenow) fragment	200 unit	EBT-1021	5 unit/㎕

Description

DNA Polymerase I Large (Klenow) Fragment is a proteolytic product of *E. coli* DNA Polymerase I which retains polymeri-zation and 3′→5′exonuclease activity, but has lost 5′→3′exonuclease activity. Klenow fragment retains the polymerization fidelity of the holoenzymewithout degrading 5′termini. Klenow Fragment can be used as follows

- Fill-in of 5′overhangs to form blunt ends
- Removal of 3′overhangs to form blunt ends
- Second strand cDNA synthesis
- Second strand synthesis in mutagenesis protocols

5′→ 3′ Polymerase

dNTPs

5′... GCT-3 → 5′... GCTAGCT-3′

3′... CGATCGA-5′ 3′... CGATCGA-5′

3′→ 5′ Exonuclease

5′... GCACG-3′ → 5′... GC-3 ′

3′... CA-5′ 3′... CA-5 ′

Storage Buffer

5 unit/μl in 100 mM KPO_4, pH 6.5, 1 mM DTT, and 50% glycerol.

Unit Definition

One unit is defined as the amount of enzyme required to convert 10 nmole of dNTP to an acid-insoluble form in 30 min at 37°C.

10x Reaction Buffer

500 mM Tris-HCl, pH 7.2, 100 mM $MgSO_2$, 1 mM DTT.

Klenow Fragment is also active in any restriction enzyme reaction buffer and T4 DNA Ligase reaction buffer when supplemented with dNTPs.

QC tests

Activity, SDS-PAGE purity, performance tests, DNase.

Storage Condition

Store at -20℃.

Heat Inactivation

75°C for 10 min.

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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 72°C for 10 min 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 12°C for 10 minutes.
3) Add 5μl of the 1mM dNTP mixture to the DNA.
4) Incubate at 37°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.