



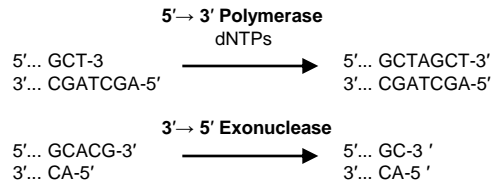
DNA Polymerase I Large (Klenow) fragment

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

Description

DNA Polymerase I Large (Klenow) Fragment is a proteolytic product of *E. coli* DNA Polymerase I which retains polymerization and 3'→5' exonuclease activity, but has lost 5'→3' exonuclease activity. Klenow fragment retains the polymerization fidelity of the holoenzyme without 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



Storage Buffer

5 unit/ μ l in 100 mM KPO₄, 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 MgSO₂, 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°C.

Heat Inactivation

75°C for 10 min.

Usage Information

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

- 1) Add the following components to the microcentrifuge tube :

DNA (1–5 μ g digested DNA containing 5' -overhangs)	x μ l
10x Reaction Buffer	2 μ l
1mM dNTP mix (0.25mM each)	5 μ l
DNA Polymerase I (2.5 units)	0.5 μ l
Nuclease-Free Water to final volume	20 μ l
- 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 (1–5 μ g digested DNA containing 5' -overhangs)	x μ l
10x Reaction Buffer	2 μ l
DNA Polymerase I (2.5 units)	0.5 μ l
Nuclease-Free Water to final volume	20 μ l
- 2) Incubate at 12°C for 10 minutes.
- 3) Add 5 μ l 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 10-100 μ l volume.

Nucleotide mix prepared by mixing equal volumes of the 3 unlabeled 300 μ M nucleotides chosen minus the nucleotide selected as label.

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

Nucleotide mix	10 μ l
Nick translation 10X buffer	5 μ l
Sample DNA (at 0.2 μ g/ μ l)	5 μ l
[α - ³² P]dCTP (400Ci/mmol at 10mCi/ml)	7 μ l
DNA Polymerase I/DNase I mix.	5 μ l
Nuclease-Free Water to final volume	50 μ l
- 2) Incubate at 37°C for 1 hour.
- 3) Add 5 μ l stop solution (0.25M EDTA (pH 8.0)).

Nick translation 10X buffer

500mM Tris-HCl (pH 7.2)
100mM MgSO₄
1.0mM DTT

DNA Polymerase I/DNase I mix

50% glycerol
50mM Tris-HCl (pH 7.2)
10mM MgSO₄
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.