Klenow Fragment of DNA Polymerase I

Catalogue number: MB00901, 300 U

Description

Klenow Fragment of DNA Polymerase I is a truncated fragment of *E. coli* DNA polymerase I that lacks the 5' → 3' exonuclease activity while retaining the 3' → 5' exonuclease activity (proofreading activity). The enzyme is well suited for use in 3´-end labelling of DNA fragments for sequence analysis, for the creation of blunt-ends by the filling-in of 5´-overhangs or by the removal of 3´-overhangs, for second-strand synthesis of cDNA and for random primer labelling of DNA fragments.

Storage temperature

Klenow Fragment of DNA Polymerase I should be stored at -20 °C in a constant temperature freezer. The protein will remain stable till the expiry date if stored as specified.

Unit definition

One unit of enzyme activity is defined as the amount of enzyme that will incorporate 10 nmol of total nucleotides into an acid-insoluble material in 30 min at 37 °C.

Enzyme concentration: 5 U/µL

Inactivation

Klenow Fragment of DNA Polymerase I is heat inactivated at 75 °C for 10 min.

System components and Reaction Conditions

Klenow Fragment of DNA Polymerase I is provided with a dedicated highly optimized NZYtech reaction buffer and displays an optimum temperature of 37 °C.

Standard protocol for blunt ends formation

The following standard protocol serves as a general guideline for blunting ends by 3' overhang removal and 3' recessed (5' overhang) end fill-in using Klenow Fragment of DNA Polymerase I. Preferably the enzyme should be added last.

1. Prepare the following 50 µL reaction:

Component	Volume
Substrate DNA	≤ 1 µg
Klenow reaction buffer (10x)	5 µL
dNTPs (2 mM) (not provided)	1 µL
Klenow DNA polymerase	1 µL (5 U)
Nuclease-free H_2O (Cat. No. MB11101)	up to 50 µL

Note: *Precautionary care should be taken to avoid create recessed ends due to the 3´ → 5´ exonuclease activity of the enzyme; for this, avoid elevated temperatures, excessive amounts of enzyme or long reaction times as well as use appropriate amounts of dNTPs.*

2. Gently mix and pulse.

3. Incubate at 25 °C for 15 minutes.

4. Stop reaction by adding EDTA to a final concentration of 10 mM and heating to 75°C for 10 minutes.

5. To obtain a highly pure product, perform a column purification step using NZYGelpure kit (Cat. No. MB011). Best results may be achieved by separating cleaved DNA through agarose gel electrophoresis prior to DNA clean-up.

Quality control assays

Purity

Recombinant Klenow Fragment of DNA Polymerase I is >95% pure as judged by SDS polyacrylamide gel electrophoresis followed by BlueSafe (NZYtech, Cat. No. MB152) staining.

Nuclease assays

0.2-0.3 µg of pNZY28 plasmid DNA are incubated with 5 U of Klenow Fragment of DNA Polymerase I in 1× Reaction buffer for 14-16 hours at 37 °C. Following incubation, the DNA is visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the DNA.

Functional assay

Klenow Fragment of DNA Polymerase I is tested for activity in a reaction to create blunt-ends in a plasmid DNA containing 3'- and 5´-overhangs. After conversion, the vector is re-ligated using T4 DNA Ligase (NZYtech, Cat. No. MB007). The efficiency of Klenow´s reaction is evaluated by the number of bacterial colonies transformed with the re-ligation product.

V2401

For research use only.

Estrada do Paço do Lumiar, Campus do Lumiar - Edifício E, R/C, 1649-038 Lisboa, Portugal Tel.: +351.213643514 Fax: +351.217151168

www.nzytech.com