NZYtech
genes & enzymes

Klenow Fragment (3′ → 5′ exo–)

Catalogue number: MB42101, 200 U

Description

Klenow Fragment (exo–), corresponds to the large (Klenow) fragment of *E. coli* DNA polymerase I, which due to truncation lacks 5’ → 3’ exonuclease activity, and that was mutated to inactivate the inherent 3' → 5’ proofreading activity, while retains polymerase activity. The enzyme displays moderate strand-displacement activity and is of particular interest to generate probes using random primers, dideoxy sequencing and random priming labelling. The enzyme is also effective to fill-in 5'-overhangs of dsDNA. Note that Klenow Fragment (exo–) is not recommended for DNA blunting reactions prior to DNA ligation since it frequently adds one or more extra nucleotides to the 3'-terminus of blunt-end DNA substrates in a non-template directed fashion.

Storage conditions

Klenow Fragment (exo–) 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 dNTP into acid insoluble material in 30 minutes at 37°C.

Enzyme concentration: 5 U/ µL

Inactivation

Klenow Fragment (exo–) is heat inactivated at 75°C for 20 min.

System components and Reaction conditions

Klenow Fragment (exo–) is provided with a dedicated highly optimized NZYtech reaction buffer and displays an optimum temperature of 37 °C.

A-tailing protocol

The following standard protocol serves as a general guideline to use Klenow Fragment (exo–) to create an “A” overhang in the 3'-end of a blunt, dsDNA molecule. Preferably the enzyme should be added last.

1. Prepare the following 50 µL reaction:

Component	Volume
Purified fragment DNA	1 – 5 µg
Klenow Fragment (exo–) reaction buffer (10x)	5 µL
dATP (10 mM)	0.5 µL
Klenow Fragment (exo–)	3 µL (15 U)
Nuclease-free H_2O (Cat. No. MB11101)	up to 50 µL

Note: *It may be required to titrate the enzyme or test different incubation periods for more effective results.*

2. Gently mix and pulse.

3. Incubate at 37 °C for 30 minutes.

4. 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

Klenow Fragment (exo–) is >95% pure as judged by SDS polyacrylamide gel electrophoresis followed by BlueSafe staining (NZYtech, Cat. No. MB15201).

Nucleases assays

To test for DNase contamination, 0.2-0.3 µg of supercoiled pNZY28 plasmid DNA are incubated with 5 U of Klenow Fragment (exo–), for 14-16 hours at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with 5 U of Klenow Fragment (exo–), for 1 hour at 37 °C. Following incubation, the nucleic acids are visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the nucleic acids.

Functional assay

Klenow Fragment (exo–) is assayed in a A-tailing reaction.

V2401

For life science research only. Not for use in diagnostic procedures.

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