T4 DNA Polymerase

Catalogue number: MB42201, 150 U

Description

T4 DNA Polymerase is a typical non-thermostable DNA polymerase displaying a much higher proofreading 3´→5´ exonuclease activity when compared with DNA Polymerase I (*E. coli*). Unlike *E. coli* DNA Polymerase I, T4 DNA Polymerase does not have a 5´→3´ exonuclease activity. The enzyme is of particular interest for 3´ overhang removal or 5´ overhang fill-in to form blunt ends. T4 DNA Polymerase does not display strand-displacement activity.

Storage conditions

T4 DNA Polymerase 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: 3 U/ µL

Inactivation

T4 DNA Polymerase is heat inactivated at 75°C for 20 min.

System components and Reaction conditions

T4 DNA Polymerase is provided with a dedicated and highly optimized NZYtech reaction buffer. The enzyme displays an optimum temperature of 37 °C, although it performs well at temperatures ranging from 12 °C – 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 T4 DNA Polymerase. Preferably the enzyme should be added last.

1. Prepare the following 50 µL reaction:

Component	Volume
Substrate DNA	≤ 1 µg
T4 DNA Polymerase reaction buffer (10x)	5 µL
dNTPs (10 mM) (not provided)	0.5 µL
T4 DNA Polymerase	1 µL (3 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 12 °C for 15 minutes.
4. Stop reaction by adding EDTA to a final concentration of 10 mM and heating to 75°C for 20 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

T4 DNA Polymerase 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 3 U of T4 DNA Polymerase for 14-16 hours at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with 3 U of T4 DNA Polymerase 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

T4 DNA Polymerase is assayed in a typical DNA fragment blunt-ending reaction.

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