T5 Exonuclease

Catalogue number: MB43301, 1000 U

Description

T5 Exonuclease is a typical DNA nuclease that functions in the 5´ to 3´ direction and attacks both single and double-stranded DNA. The enzyme substrate is the 5´-termini of a DNA molecule in the context of single or double-stranded nucleic acids as well as gaps and nicks of linear or circular double-stranded DNA. However, T5 Exonuclease does not degrade supercoiled double-stranded DNA. Thus, the enzyme may be used to remove denatured DNA generated during the alkaline-lysis protocols for plasmid purification or linear and circular DNA from supercoiled plasmid preps. It may also be used to remove incomplete ligation products from ligated circular double-stranded DNA.

Storage conditions

T5 Exonuclease 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 required to cause the change of 0.00032 A260 nm/min at 37 °C in the enzyme dedicated buffer in a total reaction volume of 50 µL.

Enzyme concentration: 10 U/ µL

Inactivation

T5 Exonuclease is highly resistant to heat inactivation. Thus, alternative protocols should be considered when requiring removing the enzyme from reactions, such as DNA silica column purification or phenol/chloroform extraction.

System components and Reaction conditions

T5 Exonuclease is provided with a dedicated and highly optimized NZYtech reaction buffer and displays an optimum temperature of 37 °C.

Standard protocol

The following standard protocol serves as a general guideline to degrade single-stranded or double-stranded nicked or linear DNA with T5 Exonuclease (the enzyme does not attack supercoiled dsDNA). Preferably the enzyme should be added last.

1. Prepare the following 50 µL reaction:

Component	Volume
Substrate DNA	≤ 1 µg
T5 Exonuclease reaction buffer (10x)	5 µL
T5 Exonuclease	1 µL (10 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 specific results or partial digestions.*

2. Gently mix and pulse.
3. Incubate at 37 °C for 30 minutes.
4. If required, stop the reaction by adding EDTA to at least 15 mM final concentration.
5. To obtain a highly pure product, perform a column purification step using NZYGelpure kit (NZYtech, 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

T5 Exonuclease 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 10 U of T5 Exonuclease for 14-16 hours at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with 10 U of T5 Exonuclease 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

T5 Exonuclease is assayed in a reaction containing 100 ng of a double stranded DNA fragment of 1 kb under standard conditions. Activity is measured by monitoring reduction of A260 over time.

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