Topoisomerase I (*E. coli*)

Catalogue number: MB43501, 100 U

Description

Topoisomerase I (*E. coli*) catalyses the relaxation of negatively supercoiled DNA as the enzyme has been implicated in the knotting and unknotting double-stranded DNA. In addition, the enzyme is involved in the linking of complementary rings of single-stranded DNA into double-stranded rings.

Storage conditions

Topoisomerase I (*E. coli*) 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 catalyses the relaxation of >95% of 0.5 µg of negatively supercoiled pNZY28 plasmid DNA in a total reaction volume of 25 µL in 15 minutes at 37 °C. DNA supercoiling is assessed by agarose gel electrophoresis.

Enzyme concentration: 5 U/ µL

Inactivation

Topoisomerase I (*E. coli*) is heat inactivated at 65 °C for 20 min.

System components and Reaction conditions

Topoisomerase I (*E. coli*) 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 process supercoiled double-stranded DNA relaxation by nicking using Topoisomerase I (*E. coli*). Preferably the enzyme should be added last.

1. Prepare the following 50 µL reaction:

Component	Volume
Substrate DNA	≤ 1 µg
Topoisomerase I reaction buffer (10x)	5 µL
Topoisomerase I	1 µL (5 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 15 minutes.

4. If required, inactivate the reaction at 65 °C for 20 min.

Quality Control Assays

Purity

Topoisomerase I (*E. coli*) 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 Topoisomerase I (*E. coli*) for 14-16 hours at 37 °C. Following incubation, the nucleic acid is visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the nucleic acid.

Functional assay

Topoisomerase I (*E. coli*) is assayed in a reaction containing 500 ng of negatively supercoiled pNZY28 plasmid DNA under standard conditions. Activity is measured by checking plasmid DNA relaxation through agarose gel electrophoresis.

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