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# DNA Topoisomerase I (Vaccinia Virus)

**Product Number: DB004**

## Shipping and Storage

Low temperature transportation; Shelf life of one year at -20℃.

## Components

| Components | 1000U |
|---|---|
| DNA Topoisomerase I (10U/μL) | 100μL |
| 10X Topo Reaction Buffer | 1mL |

## Description

The DNA topoisomerase I produced by our company is a type I eukaryotic topoisomerase derived from the smallpox virus. The DNA Topoisomerase I has DNA Relaxing activity, which can cause double stranded covalently closed circular positive or negative supercoiled DNA to undergo supercoiling, thereby forming double stranded circular DNA molecules with fewer positive or negative supercoils. Vaccinia DNA Topoisomerase I can also cause double stranded DNA to form knots or undo knots, resulting in complementary single stranded circular DNA becoming double stranded circular DNA. In addition, Vaccinia DNA Topoisomerase I can specifically recognize the 5 '-... (C/T) CCTT... -3' sequence in double stranded DNA, and can open a phosphodiester bond on one strand after the 5 '-... (C/T) CCTT ↓ sequence. The energy released by breaking the phosphodiester bond can catalyze the 3' phosphate group at the DNA incision to form a covalent ester bond with the 274th tyrosine (Try) hydroxyl group of topoisomerase, thus forming a covalent complex between DNA and the enzyme. The covalent ester bond formed simultaneously can be attacked by the 5 'hydroxyl group of the cleaved single stranded DNA, and without the need for ATP and DNA ligase, the previously formed phosphodiester bond can be restored, that is, the DNA can be reconnected while releasing Vaccinia DNA Topoisomerase I.

## Application

At present, DNA Topoisomerase I (vaccine virus) is often used as a novel tool enzyme for DNA recombination and ligation, for the ligation, defect repair, and linker ligation of DNA vectors and recombinant fragments.

## Features

1. Features: DNA supercoiling and specific opening of phosphodiester bonds.
2. Source: Escherichia coli strains carrying the Vaccinia virus topoisomerase gene; Its molecular weight is about 37KDa;
3. Enzyme activity definition: The amount of enzyme required to completely unwind 1μg of pUC19 carrier after incubation at 37℃ for 30 minutes is defined as one enzyme activity unit.
4. Purity and concentration: SDS-PAGE detection shows a purity of ≥95%; Endogenous nucleic acid residue<1pg/μL (qPCR detection); 10U/μL。
5. Inactivation or inhibition: Heating at 80 ° C for 20 minutes can fully inactivate DNA Topoisomerase I.
6. Enzyme storage buffer：50mM Tris-HCl，1mM DTT，0.1mM EDTA，0.1 M NaCl，0.1%Triton X-100，50%glycerol，pH 7.5.
7. 10x Reaction buffer： 500mM Tris-acetate, 1M NaCl, 25 mM $MgCl_2$, 1mM EDTA， pH 7.5.

## Protocol

1. Reference reaction system:

| DNA | 1μg |
|---|---|
| 10X Topo Reaction Buffer | 2μL |
| DNA Topoisomerase I (10U/μL) | 0.1μL |
| Nuclease-Free water | To 20μL |

2. After setting up the above system, gently mix well, and then centrifuge the liquid to the bottom of the tube.
3. Incubate at 37 ℃ for 30 minutes, and after the reaction is complete, place it on ice to terminate the reaction.
4. Agarose gel without ethidium bromide (EB) should be used for agarose gel electrophoresis to detect reaction products. After electrophoresis, nucleic acid staining and gel imaging should be performed.

**Note**

1. Because when the gel contains ethidium bromide, ethidium bromide will bind to the bases of DNA and catalyze the formation of a super helix. Therefore, when agarose gel electrophoresis is used to detect plasmid products treated with DNA Topoisomerase I (vaccinia virus), gel without ethidium bromide (EB) should be used for electrophoresis. After electrophoresis, ethidium bromide or other appropriate nucleic acid dyes should be used for nucleic acid staining detection of gel.
2. When using this product, it is advisable to store the enzyme in an ice box or on an ice bath. After use, it should be immediately stored at -20ºC.
3. For your safety and health, please wear lab coats and disposable gloves when operating.