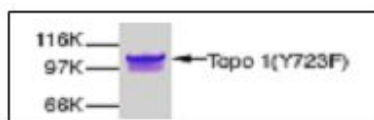


## Topo I (Y723F)

### Human DNA Topoisomerase I

#### human, recombinant, Sf9 insect cells

Cat. No.	Amount
PR-739	5 $\mu$ g



For *in vitro* use only  
Quality guaranteed for 12 months  
Store at -80°C

#### Avoid freeze / thaw cycles

#### Form

Liquid. Supplied in 20 mM Tris-HCl, pH 8.0, 100 mM KCl, 0.2 mM EDTA, 1 mM DTT, 20 % glycerol.

#### Activity

0.1-1 ng of mutated Topo I (Y723F) has been tested for relaxation activity.

#### Molecular Weight

100 kDa

#### Purity

> 95% by SDS-PAGE

#### Description

Human DNA Topoisomerase I is the best studied of the DNA topoisomerase family. It catalyzes the relaxation of both positive and negative supercoiled DNAs without the requirement of energy. In addition to DNA replication and transcriptional activation, DNA Topoisomerase I also plays a major role in pre-mRNA splicing, cell cycle, and other gene regulatory pathways during cell growth and development.

Tyrosine 723 was identified as an active site for the DNA binding activity of DNA Topoisomerase I. The covalent intermediate of topo I and DNA complex includes nucleophilic attack by the O<sup>4</sup>-oxygen of tyrosine 723 on a phosphoester linkage in the DNA. Mutation from tyrosine to phenylalanine at position 723 preferentially binds the supercoiled DNA rather than relaxed DNA in the mixture of supercoiled and relaxed DNAs. But mutation at Tyr723 neither affects its kinase activity that phosphorylates splicing factors of SR protein family nor its transcription activity of class II genes *in vitro*.

The mutant Y723F of DNA Topoisomerase I protein (Y723F) was expressed in baculovirus system and purified by using an affinity column and FPLC chromatography.

Purified Topo I (Y723F) has been tested for *in vitro* DNA relaxation assay.

Purified mutant Topo I protein (Y723F) is greater than 95% homogeneous and contains no detectable protease, DNase, and RNase activity.

#### Selected References:

- Liu (1989) DNA topoisomerase poisons as antitumor drugs. *Annu. Rev. Biochem.* **58**:351.  
Champoux (2001) DNA topoisomerases: structure, function, and mechanism. *Annu. Rev. Biochem.* **70**:369.  
Champoux (1981) DNA is linked to the rat liver DNA nicking-closing enzyme by a phosphodiester bond to tyrosine. *J. Biol. Chem.* **256**:4805.  
Madden *et al.* (1992) Overexpression of human topoisomerase I in baby hamster kidney cells: hypersensitivity of clonal isolates to camptothecin. *Cancer Res.* **52**:525.  
Lynn *et al.* (1989) Peptide sequencing and site-directed mutagenesis identify tyrosine-727 as the active site tyrosine of *Saccharomyces cerevisiae* DNA topoisomerase I. *Proc. Natl. Acad. Sci. USA* **86**:3559.  
Madden *et al.* (1996) Preferential binding of human topoisomerase I to



## Topo I (Y723F)

### Human DNA Topoisomerase I

#### human, recombinant, Sf9 insect cells

superhelical DNA. *EMBO J.* **14**:5399.

Rossi *et al.* (1998) The C-terminal domain but not the tyrosine 723 of human DNA topoisomerase I active site contributes to kinase activity. *Nucleic Acids Res.* **26**:2963.

Wang *et al.* (1998) DNA topoisomerase I and PC4 can interact with human TFIIIC to promote both accurate termination and transcription reinitiation by RNA polymerase III. *Mol. Cell* **1**:749.