

DNA Topoisomerase I, *Vaccinia*

Cat. Nos. VT710500, VT7101K, and VT7105K

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1. Introduction

DNA Topoisomerase I from *Vaccinia* virus is a 314 amino acid, 32 kD, type I eukaryotic topoisomerase that catalyzes the breakage and formation of phosphodiester bonds in a single strand of a duplex DNA molecule.¹⁻⁴ The enzyme binds to the DNA and cleaves at the 3' side of a specific target sequence [5' (C/T)CCTT↓].² Cleavage of the strand containing the recognition sequence proceeds through a transesterification reaction in which a covalent bond forms between a tyrosine residue of the protein and the 3'-phosphate group of the final thymidine of the target sequence. The other DNA strand remains intact. Religation of the original phosphodiester bond may relax the DNA, resulting in fewer positive or negative superhelical turns.

If the (C/T)CCTT recognition site is located within a few bases of the end of the molecule, the few bases 3' of the nick dissociate from the Topoisomerase I-DNA complex. The enzyme may then join the cleaved (C/T)CCTT-containing DNA with another DNA duplex to create a recombinant molecule, provided the new DNA duplex can potentially basepair with the non-cleaved strand.^{3,4} Recombinant molecules can likewise form with the 5' end of RNA molecules.⁴

DNA Topoisomerase I from *Vaccinia* is available in 500-, 1,000- and 5,000-Unit sizes at a concentration of 10 U/μl.

2. Product Specifications

Storage: Store only at -20°C in a freezer without a defrost cycle.

Storage Buffer: DNA Topoisomerase I from *Vaccinia* is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1.0 mM dithiothreitol, 0.1 mM EDTA, and 0.1% Triton® X-100.

Unit Definition: One unit of DNA Topoisomerase I from *Vaccinia* converts 1 μg of supercoiled closed circular (Form I) pUC19 DNA to relaxed closed circular form (Form II) in 1 hour at 37°C.

Quality Control: The enzyme is function-tested in a reaction containing 50 mM Tris-acetate (pH 7.5), 100 mM NaCl, 2.5 mM MgCl₂, 0.1 mM EDTA, 1 μg supercoiled pUC19 DNA, and varying amounts of enzyme for 1 hr at 37°C.

Contaminating Activity Assays: DNA Topoisomerase I from *Vaccinia* is free of detectable exo- and endonuclease and RNase activities except for the inherent endonucleolytic properties of the enzyme.

3. References

1. Shuman, S. *et al.*, (1988) *J. Biol. Chem.* **263**, 16401.
2. Shuman, S. (1991) *J. Biol. Chem.* **266**, 11372.
3. Shuman, S. (1992) *J. Biol. Chem.* **267**, 8620.
4. Sekiguchi, J. *et al.*, (1997) *J. Biol. Chem.* **272**, 15721.

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