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ProductInformation

Topoisomerase I
from vaccinia virus

Catalog Number **T9194**
Storage Temperature –20 °C

EC 5.99.1.2
Synonym: Topo I

Product Description

Topoisomerase I (Topo I) from vaccinia virus is a purified enzyme, free of detectable exo- and endonuclease and RNase activities. It has a molecular mass of 32 kDa. Topoisomerase I relaxes supercoiled DNA molecules. The enzyme initiates transient breakages and rejoins of phosphodiester bonds in superhelical turns of closed-circular DNA. Enzyme activity is independent of right- and left-handed superhelices.

Topoisomerase I plays a major role in critical cellular processes by catalyzing the breakage and religation of phosphodiester bonds in a single strand of DNA. This results in the removal of supercoils (either positive or negative superhelical turns). Topo I from vaccinia virus is a type I eukaryotic topoisomerase that cleaves DNA 3′ to the target sequence [5′(C/T)CCTT↓].[1] Cleavage of the strand containing this sequence occurs by a transesterification reaction in which a covalent bond is formed between a tyrosine on the Topo I and the 3′-phosphate of the last thymidine of the target sequence. The other DNA strand is not cleaved. Subsequent religation of the phosphodiester bond results in DNA with fewer superhelical turns. Topo I does not require Mg^{2+} to function, but low concentrations of this cation may increase its activity.

If the (C/T)CCTT site is within a few bases of the end of the DNA molecule, those bases 3′ of the nick dissociate from the Topo I-DNA complex. Topo I may then create a recombinant molecule by joining the cleaved (C/T)CCTT-containing DNA with another DNA duplex if the other DNA duplex can basepair with the noncleaved strand.[2,3] Topo I can also form recombinant molecules with the 5′-end of RNA molecules.[3]

Topoisomerase I is important in studying vital processes including replication, transcription, and recombination.[4] The enzyme may be used to study DNA structure and topology such as: the effects of supercoiling on transcription *in vitro,* chromatin reconstitution *in vitro*, and the degree of supercoiling of DNA. It can also be used to assay mutant plasmids which differ in length by only one base-pair and to increase restriction endonuclease digestion of resistant DNA substrates by unwinding the DNA coils to expose restriction sites.

Vaccinia Topoisomerase I is provided in a solution of 50 mM Tris-HCl, pH 7.5, containing 100 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.1% TRITON® X-100, and 50% glycerol.

One unit converts 1 µg of supercoiled closed circular (Form I) pUC19 DNA to relaxed closed circular form (Form II) in 1 hr at pH 7.5 at 37 °C.

Enzyme activity is increased in the presence of 2.5 mM Mg^{2+}.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store at –20 °C. Do not store in a frost-free freezer. It is recommended that the enzyme be aliquoted after the first thaw to prevent loss of activity that occurs with repeated freeze/thaw.

References

1. Shuman, S., Site-specific interaction of vaccinia virus topoisomerase I with duplex DNA. Minimal DNA substrate for strand cleavage in vitro. *J. Biol. Chem.*, **266**, 11372-113729 (1991).
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4. Krogh, B.O., et al., Effect of 3'-5' phosphodiesters on DNA transesterification by vaccinia topoisomerase. *J. Biol. Chem.*, **276**, 20907-20912 (2001).
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