Topoisomerase 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 molecularmass 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 virusis a type I eukaryotic topoisomerase that cleaves DNA ．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 targetsequence. 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 100mM NaCl, 1mM EDTA, 1mM DTT, 0.1% TRITON ．X-100, and 50% glycerol.

One unit converts 1ug 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 Mg2+.

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

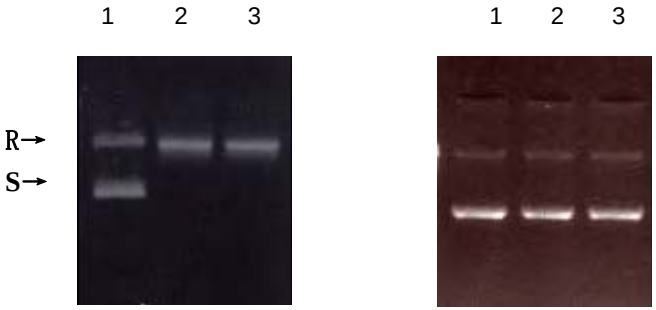
Store at –20 °C. Do not store in a frost-free freezer. It is recommended that the enzyme be aliquoted after the first thaw to prevent loss of activity that occurs with repeated freeze/thaw.

反应举例:(*超螺旋pBR322 DNA的解螺旋反应*)

1. 在微量离心管中配制下列反应液。

pBR322 RF I	0.5 μg
10X Topoisomerase I Buffer	2 μl
DNA Topoisomerase I	1 U
0.1% BSA	2 μl
灭菌蒸馏水 up to 20 μl	

2. 37℃反应30分钟。
3. 进行1% Agarose电泳，确认pBR322是否由超螺旋型转变为松散型。



A）不含EtBr的0.7% Agarose **B）含1 μg/μl EtBr的0.7% Agarose**

Lane 1：pBR322 DNA(RF I)

Lane 2：37℃，15 min

Lane 3：37℃，30 min

R：Relaxed pBR322 DNA

S：Supercoiled pBR322 DNA

References

1. Shuman, S., Site-specific interaction of vaccinia virus topoisomerase I with duplex DNA. MinimalDNA substrate for strand cleavage in vitro. J. Biol. Chem., 266, 11372-113729 (1991).
2. Shuman, S., DNA strand transfer reactions catalyzed by vaccinia topoisomerase I. J. Biol. Chem., 267, 8620-8627 (1992).
3. Sekiguchi, J., et al., Kinetic analysis of DNA and RNA strand transfer reactions catalyzed by vaccinia topoisomerase. J. Biol. Chem., 272, 15721-15728 (1997).
4. Krogh, B.O, et al., Effect of 3'-5' phosphodiesters on DNA transesterification by vaccinia topoisomerase. J. Biol. Chem., 276, 20907-20912 (2001).
5. Cheng, C., Shuman, S., DNA strand transfer catalyzed by vaccinia topoisomerase: ligation of DNAs containing a 3 ˙mononucleotide overhang. Nucleic Acids Res. 28, 1893, (2000).

Topoisomerase I

————from vaccinia virus

(Synonym: Topo I)

货号	产品名称	规格	备注
Cat：TE021	Topoisomerase I	2000U/100ul	
	10XTopo I buffer	1ml	
	0.1% BSA	1ml	

贮存条件：

低温运输： -20℃贮存，有效期 3 年。

生产日期：见包装