Topoisomerase II, Alpha

Product number 78303

Relaxation of supercoiled DNA protocol

Topoisomerase II, Alpha alters the topological state of nucleic acids by passing an intact DNA helix through a transient break which generates a separate DNA helix. As a result of its double-stranded DNA passage mechanism, the enzyme can relax negatively or positively supercoiled DNA, as well as catenate/decatenate or knot/unknot DNA molecules. Topoisomerase II, Alpha has an absolute requirement for divalent cation and ATP (or dATP).

Properties

Molecular weight: 340 kDa homodimer

Optimum pH: 7.9, rapidly loses activity below pH 7.5

Optimum temperature: 30°–37°C

Requirement for divalent cation: 5 mM Mg^{2+} is optimum for catalytic activity and 5 mM Ca^{2+} for DNA cleavage

Optimum ATP concentration: 0.5–1.0 mM

Optimum ionic strength: 100–170 mM NaCl/KCl

Inhibitors: N-ethylmaleimide, zinc, novobicin, coumermycin A_1, etoposide, amsacrine

10X Topoisomerase II Reaction Buffer

(PN 73592, included with enzyme):

100 mM Tris-HCl, pH 7.9, 500 mM NaCl, 500 mM KCl, 50 mM $MgCl_2$, 1 mM EDTA, 150 µg/ml BSA, 10 mM ATP

Topoisomerase II, Alpha and 10X Topoisomerase II Reaction Buffer (PN 73592) are functionally tested in the following protocol using 300 ng (0.1 pmol) pBR322 DNA at30°C:

Relaxation of supercoiled DNA

1. Mix

10X Topo II Reaction Buffer	2 µl
Supercoiled DNA	0.1–1.0 pmol
Topoisomerase II, Alpha	1–10 units
H_2O	to 20 µl

2. Mix and incubate at 30°–37°C for 15 minutes.
3. Stop the reaction by the addition of 3 µl of 7 mM EDTA or by the addition of SDS to 1%.

Documentation and support

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Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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