Human Topoisomerase II beta



Product Description (Product Numbers HTB201, HTB205, HTB210 and HTB220)

Human topoisomerase II beta is prepared by overexpressing the enzyme in baculovirus-infected insect cells ($Spodoptera\ frugiperda$) and purifying it by methods developed in-house. The enzyme is supplied at a minimum concentration of 10 U/µI in Dilution Buffer . Store at -80 °C.

It is recommended that the enzyme is aliquoted to avoid repeated freeze-thaw cycles.

For in vitro laboratory research use only.

Dilution Buffer

50 mM Tris.HCl (pH 7.5) 100 mM NaCl 1 mM DTT 0.5 mM EDTA 50 % (v/v) glycerol 50 µg/ml albumin

Assay Buffer (supplied as 10X stock)

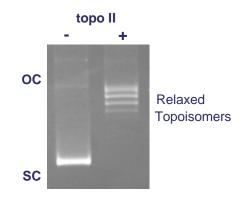
50 mM Tris.HCl (pH7.5) 125 mM NaCl 10 mM MgCl₂ 5 mM DTT 100 μg/ml albumin ATP (30X stock)

30 mM ATP

Relaxation Assay

A typical reaction will contain 3 µl of (10x) Assay Buffer, 1 µl of (30x) ATP, 0.5 µl of supercoiled pBR322 (1 µg/µl), plus human topo II, in a total volume of 30 µl. 1 U of human topo II will relax 0.5 µg of supercoiled pBR322 when incubated in 1X Assay Buffer plus 1 mM ATP in a total reaction volume of 30 µl at 37 °C for 30 minutes.

Gels should be run in the absence of ethidium bromide or chloroquine (CQ).



Quality Control

1) Purity: Human topoisomerase II is purified to > 95 % purity as judged by SDS-polyacrylamide gel electrophoresis. 2) Tests for human topoisomerase I contamination by looking for relaxation of sc pBR322 under topoisomerase I assay conditions were negative. 3) kDNA or pBR322 were also incubated for 4 hrs in assay buffer (+ 10 mM MgCl₂) at 37 °C. These tests were negative for the formation of linear products, showing the absence of nuclease contamination.