Human Topoisomerase II alpha Relaxation Assay Kit

Product Description (Product Numbers HTR201, HTR202, HTR203 and HTR204)

Human topoisomerase II 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/µl in Dilution Buffer with supercoiled DNA substrate.
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)

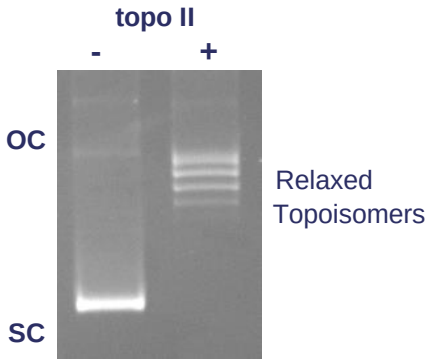
50 mM Tris.HCl (pH7.5)
125 mM NaCl
10 mM $MgCl_2$
5 mM DTT
100 µg/ml albumin

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 $MgCl_2$) at 37 °C. These tests were negative for the formation of linear products, showing the absence of nuclease contamination.