Human Topoisomerase II alpha

Product Description (Product Numbers HT201, HT205, HT210 and HT220)

Human topoisomerase II alpha is prepared by overexpression 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. However, we recommend that the enzyme is titrated into the assay to ascertain the minimum volume of enzyme required per assay to achieve full supercoiling. Particularly if the kit is being used for drug screening purposes. Please refer to the protocol for more information:

https://www.inspiralis.com/assets/TechnicalDocuments/Human-Topo-II-Alpha-Relaxation-Assay-Protocol3.pdf

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)

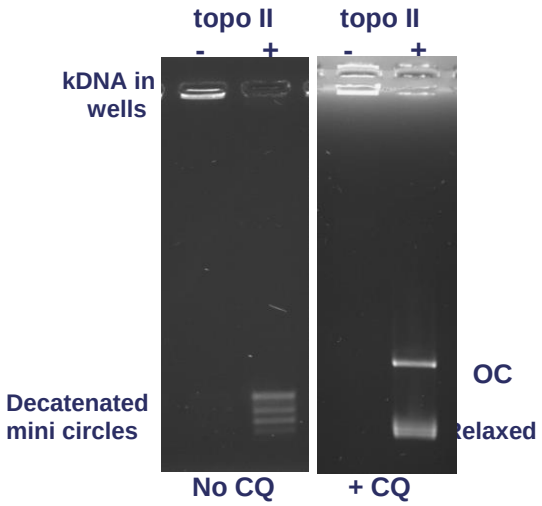
50 mM Tris.HCl (pH7.5)
125 mM NaCl
10 mM $MgCl_2$
5 mM DTT
100 µg/ml albumin

ATP (30X stock)

30 mM ATP

Decatenation Assay

A typical reaction will contain 3 µl of (10x) Assay Buffer, 1 µl of (30x) ATP, 2 µl of kDNA (100 ng/µl) plus human topoisomerase II, in a total volume of 30 µl.
1 U of topoisomerase II will decatenate 200 ng of kDNA 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 can be run in the presence or 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.

Inhibition of decatenation activity of human topoisomerase II

The inhibition of the decatenation activity of human topo II by a compound can be determined by performing the assays as described above and including a range of concentrations of the test compound. Sufficient enzyme is included to just give full decatenation in the absence of inhibitor. Initially a wide range of concentrations of inhibitor are tested and then after determining an approximation of the IC_{50}, a range of 10X to 0.1X the IC_{50} is tested. The IC_{50} for inhibition of decatenation can be visually assessed as the concentration of compound which leads to a 50% reduction in the amount of mini-circles produced. This is then verified using gel documentation software and statistical analysis. In the example below the inhibition of human topo II-catalysed decatenation of kDNA by etoposide is followed.