Human Topo II beta Decatenation Assay Kit

Product Description (Product Number HTKB201, HTKB202)

Human topoisomerase II beta is prepared by overexpressing 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.
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)

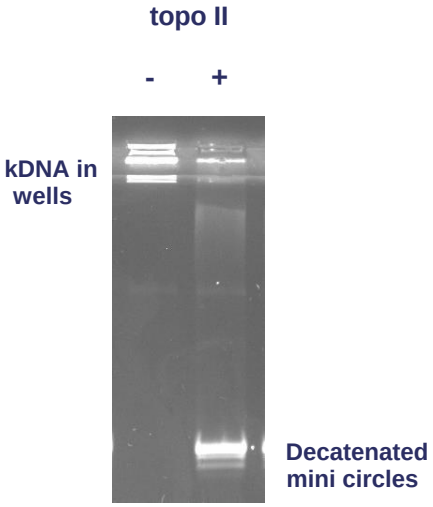
50 mM Tris.HCl (pH7.5)
125 mM NaCl
10 mM $MgCl_2$
5 mM DTT
100 µg/ml albumin

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 human 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. Reactions are stopped by the addition of an equal volume chloroform/isoamyl alcohol (24:1 v:v) and another volume of 2X stop dye (40% sucrose, 10 mM EDTA, 100 mM Tris.HCL (7.5), 0.5 ug/ml bromophenol blue).
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.