Human Topoisomerase II Alpha Decatenation Assay Kits

Product Description (Product Numbers HTK201, HTK202, HTK203 and HTK204)

Human topoisomerase II 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. However, we recommend that the enzyme is titrated into the assay to ascertain the minimum volume of enzyme required per assay to achieve full decatenation particularly if the kit is being used for drug screening purposes. Please refer to the protocol for more information: Human-Topo-II-Alpha-Decatenation-Assay-Protocol5.pdf (inspiralis.com).
Store at -80°C.
It is recommended that for larger kits 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)

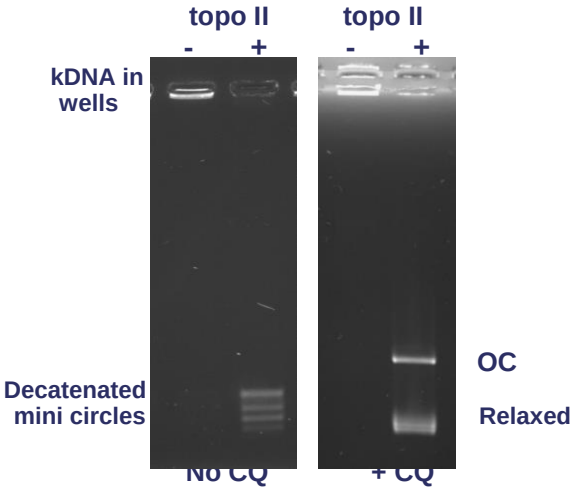
30 mM ATP

kDNA

Supplied at 100ng/µl in 10mM Tris.HCl (pH8.0), 1mM EDTA.

Decatenation Assay

1 U of topoisomerase II will decatenate 200 ng of kDNA when incubated in 1X assay buffer with 1mM 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 (EtBr) or chloroquine (CQ) which will resolve nicked open circular (OC) DNA from relaxed.



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 supercoiled pBR322 under topoisomerase I assay conditions were negative.
3) No nuclease activity was detectable under assay conditions.

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.