Human Topoisomerase II Alpha BisCat Decatenation Assay Kits

Product Description (Product Numbers HTAD201, HTAD202, HTAD203 and HTAD204)

The BisCat decatenation kits are designed to determine the decatenation activity of type II topoisomerases such as human topo II. The substrate consists of two supercoiled, singly linked minicircles of 2.3 and 2.6 Kb which are decatenated to produce two supercoiled minicircle products. It provides greater sensitivity than the standard decatenation assay which uses kDNA as the substrate. In addition, each decatenation event leads to the conversion of one substrate molecule to products which is not the case with kDNA which consists of a network of minicircles. 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 usually supplied at a minimum concentration of 10 decatenation U/µl in Dilution Buffer but see Certificate of Analysis for activity of particular lot#. 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-Protocol.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

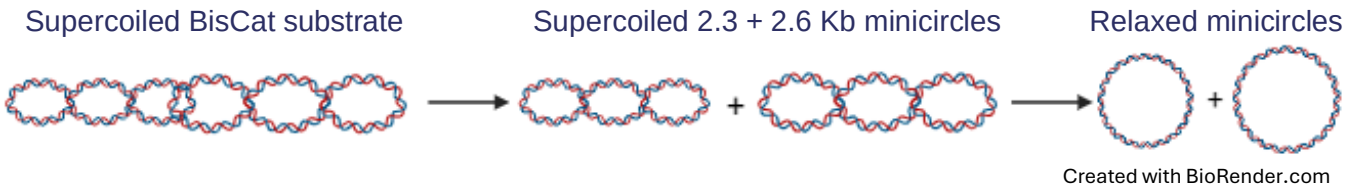
BisCat DNA

Supplied at 200ng/µl in 10mM Tris.HCl (pH8.0), 1mM EDTA.

Example Assay:

BisCat Decatenation Assay

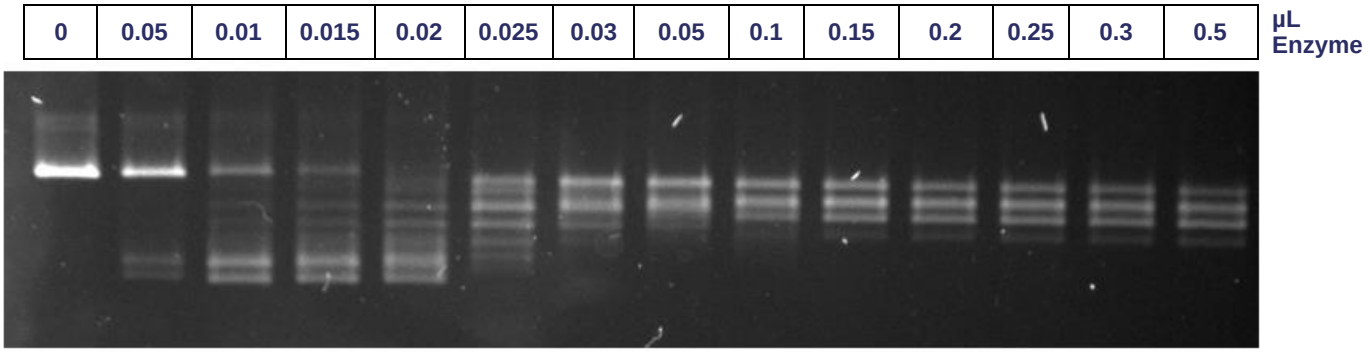
The substrate consists of two supercoiled, singly-linked minicircles of 2.3 and 2.6 Kb. Type II topoisomerases such as human topo II can decatenate the substrate to produce two supercoiled minicircle products. As more enzyme is added the minicircles are then progressively relaxed.



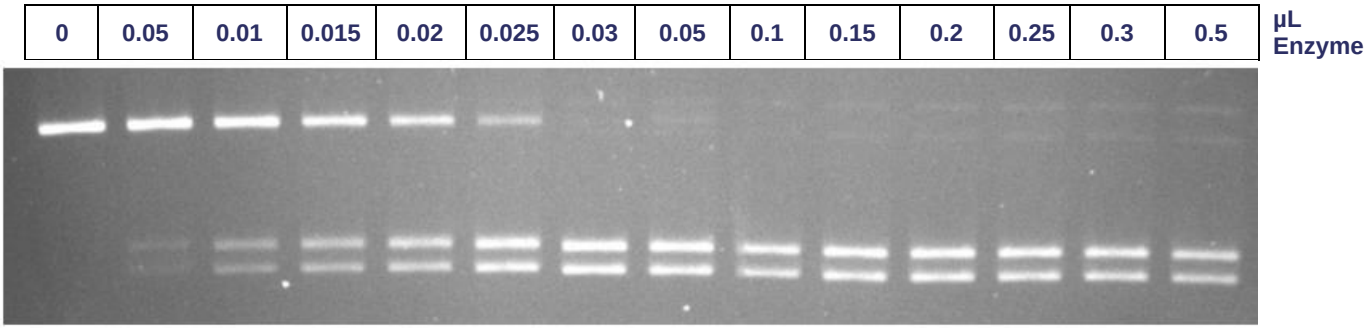
1 U of topoisomerase II will decatenate 200 ng of BisCat DNA when incubated in 1X assay buffer with 1mM ATP in a total reaction volume of 30 µl at 37°C for 30 minutes.
Gels can be run in the presence or absence of ethidium bromide (EtBr) or chloroquine (CQ) which will resolve supercoiled (SC) from relaxed DNA.

Decatenation of 200 ng BisCat DNA with increasing amounts of Human topoisomerase II alpha.

Analysis of decatenation reactions on a non-ethidium bromide containing agarose gel:



Analysis of reactions on an agarose gel containing ethidium bromide:



Result: In this example, full decatenation was achieved with the addition of 0.03 µL of human topo II alpha added, therefore the decatenation activity is 30U/µL.

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.