Manual for Topoisomerase II Assay Kit

Catalog No. 1001-1 100 Assays

Kit Description

The TopoGEN Topoisomerase II assay Kit contains reagents necessary to quantify topo II activity in crude cell extracts. Markers are included to allow unambiguous detection of topo II even in the presence of contaminating topoisomerase I. The assay is kDNA based and is specific for topo II. Nuclease activity may however cause some degradation of the kDNA substrate. Such degradation will be ATP independent. In addition, nucleases will generate a smear of degradation products in addition to linear kDNA. This kit does not contain enzyme (topo II is available on line at www.topogen.com (Catalog # 2000H-1).

Storage of Reagents

The DNAs should be stored at 4° C and buffers stored at -20° C upon receipt.

Topoisomerase II Assay Kit Description and Protocol

Summary: The kit contains all the reagents for routine detection of type II topoisomerase (topo II). DNA markers are included to allow unequivocal detection of activity. The assay highly specific since only topo II can decatenate DNA interlocking rings (topo I cannot; see Fig. 1 below).

Kit Contents: Volumes are given for the 100 assay kit size. For the 250 assay kit size, multiply volumes by 2.5 fold.

1. *Kintoplast DNA (kDNA, 20 ug total) substrate at the concentration specified in the upper right: use 0.1 to 0.2 ug per reaction.*
2. *Decatenated kDNA marker (25 ul) in gel loading buffer. Run 2-4 ul of decatenated DNA marker per gel.*
3. *Linearized kDNA marker (25 ul) in gel loading buffer; run 2-4 ul of linear marker per gel.*

**4. 10x Topo II Incomplete Assay Buffer A contains 0.5 M Tris-HCl (pH 8), 1.50 M NaCl, 100 mM $MgCl_2$, 5 mM Dithiothreitol, 300 ug BSA/ml*

**5. 10x ATP Buffer B contains 20mM ATP in water.*

*You must mix Buffers A and B together prior to make a 5x Complete Assay Buffer. To prepare a fresh stock of the 5x Assay buffer: Add equal volumes Buffer A and B to give the Complete 5x Assay Buffer (example, if you need 50 ul of 5x Complete Buffer for a single experiment, mix 25 ul of Buffer B with 25 ul of Buffer A). The Complete 5x Buffer MUST be made fresh for each experiment. Prepare only the amount needed fresh each day. DO NOT STORE THE 10x COMPLETE ASSAY BUFFER, IT IS NOT STABLE.

6. *5x Stop Buffer/gel loading dye (600 ul): 5x buffer is 5% Sarkosyl, 0.125% bromophenol blue, 25% glycerol.*

Assay Protocol: Reaction volumes should be 20-30 ul final volume (limited by volume that can be loaded onto the gel). Reactions are assembled in microfuge tubes with water, buffer and substrate kDNA. The test fractions should be added last and the reactions incubated at 37°C for 15-30 min. (or longer as appropriate) then terminated with 1/5 volume of the stop buffer. Place on ice until ready to load agarose gel.

Sample reaction (20 ul, order of addition is shown):

Distilled water---------- 16 ul (Vary as needed to bring volume up to 20 ul final)
5x Complete Assay Buffer---------- 4 ul (made fresh each day using Buffer A and Buffer B, see above)
KDNA---------- 1ul
Test Extract---------- Variable (as needed, take care not to overload with salt from extract)

- *Incubate 30 min or longer at 37°C.*
- *Add 4 ul stop loading dye*
- *Add proteinase K to 50 ug/ml, digest for 10-30 min at 37°C (optional step).*
- *Load a 1% agarose Ethidium Bromide gel (include EB at 0.5 ug/ml in Gel and 1xTAE buffer. (50x TAE buffer: 242 g Tris base, 57.1 ml glacial acetic acid, 100 ml 0.5M EDTA). (Caution, EB is mutagenic, be sure to wear gloves.)*
- *Electrophorese until dye travels about 5 cm, remove from gel box (wear gloves).*
- *Destain (distilled water) for 10-30 min room temperature.*
- *Photograph using UV transilluminator.*

Analysis of reaction products by electrophoresis: For each gel, run linear and decatenated kDNA markers (usually 2 -3 ul is sufficient for each marker). The marker DNAs are already in loading buffer. Also run a reaction without protein to mark the position of catenated kDNA substrate (should be retained in the well due to its large size). Note that extensive washing and handling of the gel after electrophoresis may dislodge catenanes from the wells; however, this does not affect the results.

Important note regarding kDNA.

With time the kDNA substrate may spontaneously release some decatenated (breakdown) products (this is normal). These released products can easily be checked by running a minus extract control lane and looking for decatenated kDNA. Degradation is more severe with aging or freeze/thawing and we recommend storage at 4°C. A small amount of decatenation is acceptable as long as a minus enzyme control is included (a kDNA only lane). Topo II activity should yield a clear increase in the decatenation products. Note also that kDNA is a mixture of nicked, open circular DNA and covalently closed (supercoiled) minicircles; thus, upon decatenation with topo II, you will often detect both minicircle species (see gel below).

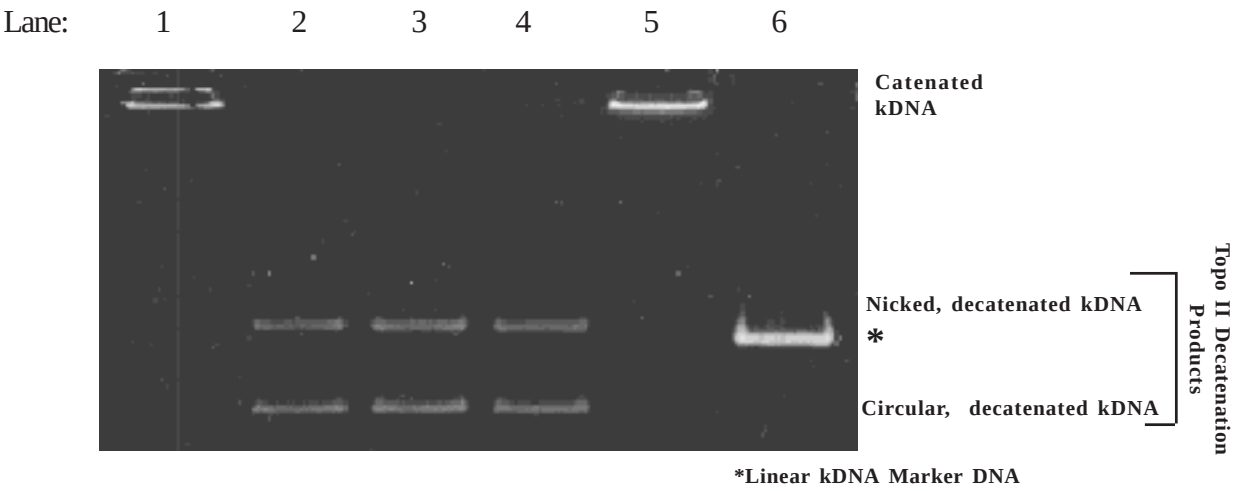

Figure 1. Purified Topo II (catalog # 2000H-1, sold separately) was incubated with KDNA for 15 min at 37°C using the assay buffer supplied with the kit. Reactions were terminated using stop buffer and loaded directly onto a 1% agarose gel containing ethidium bromide (0.5ug/ml). After electrophoresis, the gel was destained for 30 min and photographed. Note that the decatenated products contain open circular (upper band) and covalently closed circular (relaxed) minicircle DNA as marked on the right of the gel. Note also that linear DNA migrates between the nicked and relaxed species. The nicked minicircular DNA is present in all KDNA preparations. Additionally, the amount of nicked DNA may vary between KDNA preparations. Linear and decatenated KDNA markers are supplied in the kit. It is important to run these markers to identify positions of different forms. Lane 1 is a kDNA marker showing the position of catenated kDNA. Lanes 2-4 each contained 2 units of topo II (incubated for 1 hour at 37°C). Lane 5 is a reaction with purified topo I. Lane 6 is linear kDNA marker produced by digestion of kDNA with Xho I.

References:

Miller et al., J. Biol. Chem. 256:9334-9339 (1981)
Muller et al., Biochemistry 27: 8369-8379 (1988)

Other Helpful Hints

•Topo II activity can easily be extracted from nuclei using the procedure described on the TopoGEN web site: http://www.topogen.com/html/enzyme_extracts.html.
•The degree of decatenation is proportional to topo II concentration and length of incubation. Thus, if too much topo II activity is present, all of the kDNA may be decatenated. Shorter incubation times should be used in this case.
•KDNA is a collection of interlocking mini (2.5 kb) and maxicircles (ca. 8 kb). The minicircles are the predominant DNA species. Note that the minicircles exist as intact rings (covalent closed, circular) and as nicked circles. These forms will resolve out in an ethidium bromide gel (see sample gel Fig. 1).

Complications

The most serious complications arise when there are interfering proteins or substances in the extract being assayed. Crude, cell free extracts may contain excessive amounts of DNA binding proteins or positively charged proteins that stick to the DNA and inhibit enzyme access. Also, nuclease contaminants may degrade or nick the KDNA substrate and therefore obscure the results. A good way to deal with this problem includes cleaning up crude extracts by ammonium sulfate precipitation followed by column chromatography. Also, by diluting extracts and/or adding a tRNA carrier (to compete basic proteins), one can sometimes minimize such problems. When assaying crude extracts, it is also important to realize that extracts often may contain UV fluorescing contaminants (RNA or DNA breakdown products for example). The markers will help in identifying such artifacts; however, we advise that you also run one lane of protein extract *without* KDNA substrate to reveal these contaminants. Finally, high concentrations of di- or monovalent ions (for example from the extract) can mask the topo II activity. Be sure to consider the influence of salt (NaCl or KCl) from the extract when assaying topo II containing fractions (do not let the total salt concentration exceed 250-300 mM).