TopoGEN

Products and Services for **Cancer Research.**

DNA Gyrase Assay Kit
USER MANUAL

Overview:

This kit contains all reagents for routine detection of DNA Gyrase (bacterial topoisomerase II). The assay is specific for type II topoisomerases (type I activities will not be detected by this assay); however, heavy contamination by nucleases may interfere (nuclease contamination will be visible as an ATP independent reaction; whereas gyrase requires ATP). Gyrase will decatenate kDNA resulting in supercoiled monomers.

Shipping and Storage of Reagents

The buffers and DNAs should be stored at 4° C. Avoid freeze/thaw cycles of the 5x gyrase buffer since this may inactivate the ATP and can accelerate degradation of the kDNA networks.

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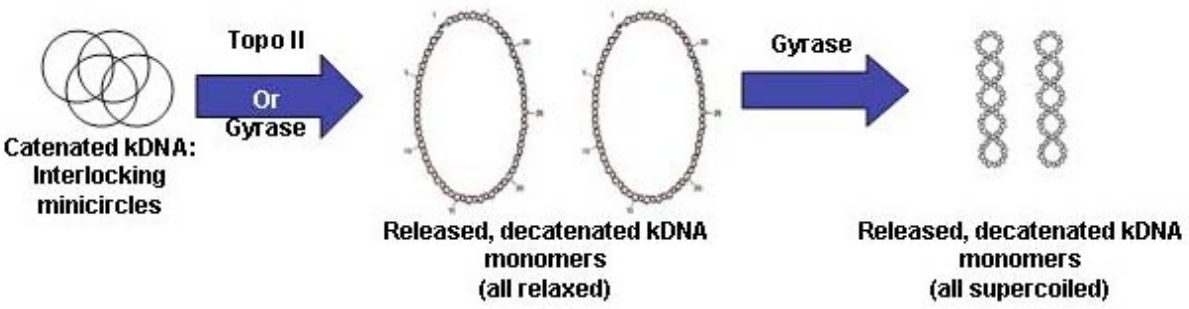
Kit Description

This assay kit is designed to allow quick and specific detection of DNA gyrase. This kit facilitates the purification and characterization of type II enzymes (DNA gyrase) and contains all reagents necessary for routine assays of type II topoisomerases that either have or do not have the ability to supercoil. The gyrase assay is based upon a two- step process: 1) decatentation of kinetoplast DNA (kDNA); 2) supercoiling of the resulting decatenated monomer kDNA species (see diagram below).



Reaction products are resolved using a novel gel system developed by TopoGEN that allows extremely rapid and unambiguous detection of gyrase activity. The appropriate buffers, DNA substrates and DNA markers are included. Purified gyrase is not included in this kit; however, decatenated and linear DNA markers are included to allow clear and facile identification of products in the extracts or fractions defined by the user. The kDNA assay will detect poisons that stimulate DNA cleavage by gyrase as well as agents that simply inhibit catalytic activity. The advantage of kDNA-based assays is that the assays are fast and easy to allow activity guided purification of inhibitors from crude mixtures or extracts.

B. Kit Contents (100 assay kit size)

1. kDNA (20 ug total) substrate at the concentration specified on the tube provided. Typically one should use 0.1 to 0.2 ug per reaction.

2. Decatenated kDNA marker (25 ul) in gel loading buffer. Run 2 ul of decatenated DNA marker per gel.

3. Linearized kDNA marker (25 ul) in gel loading buffer; run 2 ul of linear marker per gel.

4. 5x Gyrase Assay Buffer (600 ul). 1x buffer contains 35 mM Tris-HCl (pH 7.5), 24 mM KCl, 4 mM $MgCl_2$, 1 mM ATP, 2 mM Dithiothreitol, 1.8 mM Spermidine, 6.5% glycerol, 100 ug/ml BSA.

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

C. 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	14 ul (Vary as needed to bring volume up to 20 ul final)
5x Assay Buffer	4 ul
KDNA	1 ul (vary as needed to give 100-200 ng)
Test Extract	1 ul (vary as needed, take care not to overload with salt from extract, see below)

•Incubate 30-60 min or longer at 37° C.
•Add 1/5 vol of stop buffer/loading dye.
•Add proteinase K to 50 ug/ml, digest for 10-30 min at 37° C (optional step).
•Add 20 ul of Chloroform:isoamyl alcohol (24:1 mixture), vortex briefly, withdraw blue (aqueous) phase (Chloroform extraction is an 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.) Be sure EB is included in gel AND buffer.
•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. Degra- dation 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). Gyrase should yield a clear increase in the supercoiled, circular DNA decatenation products. Since kDNA is a mixture of nicked, open circular DNA and covalently closed (supercoiled) minicircles, you will often detect both minicircle species (see gel below).

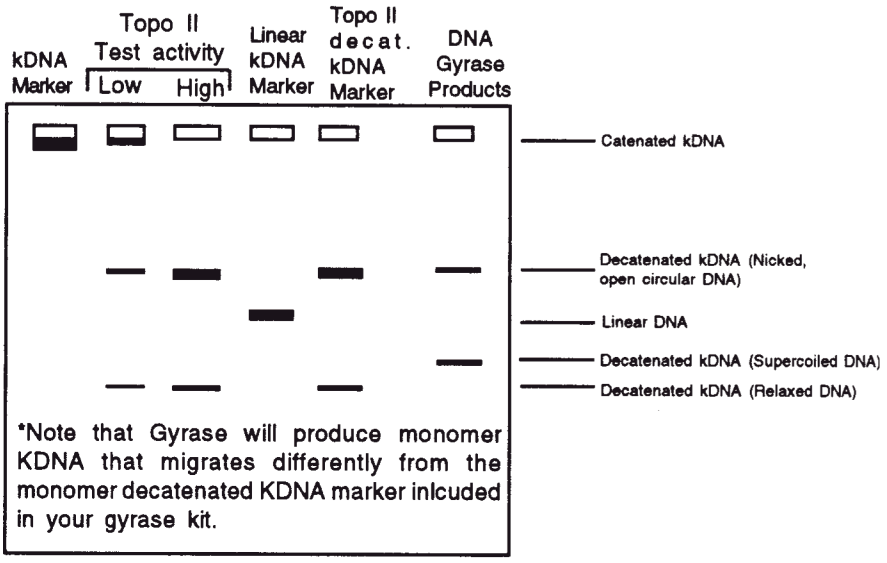


Figure 1. Schematic of Gyrase Assay kit results. Both gyrase and eukaryotic topo II have the ability to decatenate kDNA. Topo II cannot supercoil the monomeric kDNA forms; thus, the covalently closed circular monomers will migrate as a relaxed kDNA minicircle. Gyrase can supercoil these circular decatenation products and yield a slower migrating monomeric kDNA product (in ethidium bromide gels) as shown in the above figure. The nicked monomeric kDNA cannot be supercoiled since it cannot retain gyrase induced supercoils (due to the rotation around the nick site).

Other Points:
•KDNA is a collection of interlocking mini (2.5 kb) and maxicircles (ca. 8 kb). The minicircles are the predomi- nant 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 mini- mize 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 identi- fying such artifacts; however, we advise tt you also run one lane of protein extract *without* KDNA

substrate to reveal these contaminants. Note also that high concentrations of di- or monovalent ions (for example from the extract) can affect gyrase activity. Be sure to consider the influence of salt (NaCl or KCl) from the extract when assaying gyrase containing extracts or fractions (do not let the total salt concentration exceed 250-300 mM).

Any further questions or comments, please feel free to contact us:
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