

Topoisomerase II Drug Screening Kit Cat# 1009

Kit Description:

Topo II inhibitors have been shown to possess effective antibacterial, antiviral and anticancer activity. Identification of topo II inhibitors and antagonists requires tractable biochemical assays employing purified enzyme. The TopoGEN Drug Screening Kit contains the reagents necessary to screen for agents that affect enzymatic activity. To allow the customer maximum flexibility, purified topoisomerase II may be purchased separately from the kit (Catalog number 2000H-1 and 2000H-2). This kit will allow detection of two classes of topoisomerase inhibitors: those that stimulate formation of cleavable complexes and those that antagonize topo II action on the DNA. Also included in this kit is detailed documentation on design and interpretation of inhibition data. Known inhibitors are included as controls. The DNA substrate (pRYG) included in this assay is ideal for these studies because it contains a single, high affinity topo II cleavage site. Recent studies have shown that topo II cuts pRYG primarily at a single site at the 54 bp of alternating purine/pyrimidine DNA. Given that cleavage analyses require larger amounts of enzyme compared to catalytic assays, it is possible to conserve on enzyme by using a DNA substrate with a high affinity site. The assay system is based upon evaluating the formation of DNA cleavage products which may be nicked, open circular DNA or linear DNA. The products are then resolved by ethidium bromide gel electrophoresis as described in the kit.

***Kit Contents: (representative of 100 assay kit size)**

- Supercoiled DNA [pRYG DNA] 25 ug.
- Markers are linear pRYG DNA and open circular DNA
- 10X Topoisomerase II assay buffer components*

*NEW: To improve stability, the 10x Topo II Assay buffer is provided as two components: Buffer A (no ATP) and Buffer B (ATP). The Complete 10x Assay buffer is made fresh each time by combining Buffer A and B.

- Sodium dodecyl sulfate (10%).
- 10X Gel loading buffer (bromophenol blue, glycerol).
- Control inhibitor, Etoposide.
- Detailed instruction manual.

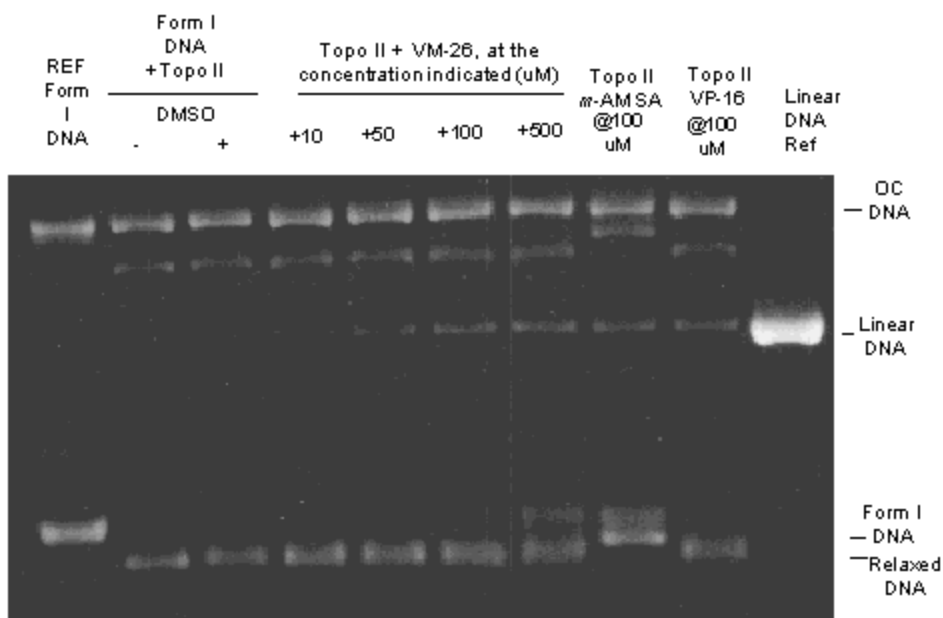
Note: Enzyme is sold separately.

Shipping/Storage Conditions: This kit is shipped at ambient temperature; storage is at -20 C.

This is a cleavage based detection kit that will reveal whether a particular agent either antagonizes the action of topo II or affects the cleavage/relication cycle. Reactions are assembled in microcentrifuge tubes using reagents supplied in the kit (buffer, DNA, control inhibitor). This kit is designed to work with purified topoisomerase II (see below) provided by TopoGEN, Inc. (sold separately). After adding enzyme, the reactions are incubated 15-30 min (37°C), and terminated by addition of SDS.

A typical reaction:

H₂O: Volume variable, make up to volume (20 ul in this case)
 10X Assay buffer 2 µl (made fresh from Buffers A and B, see Kit details)
 DNA (supercoiled) 1 µl
 Test Compound or control inhibitor: Variable (limited by solvent effects as addressed in controls)
 **Purified Topoisomerase II: 4-6 Units (enzyme sold separately)
 Incubate 15-30 minutes at 37°C
 Add 2 µl SDS; digest with proteinase K (50 ug/ml)
 Add 0.1 vol sample loading dye (supplied)
 Load gel: 1% agarose containing 0.5 ug/ml ethidium bromide along with the marker DNAs included in the kit.



The reactions indicated above each lane in the Figure were carried out using the enzyme provided by TopoGEN (cat. number 2000H-1) and 1 ul of DNA (0.25 ug of form I pRYG, as provided in each kit). The reaction products were analyzed on a 1% agarose gel; the running buffer contained 0.5 ug/ml ethidium bromide. The gel was then destained in distilled water prior to photodocumentation. Formation of the cleavable complex (linear DNA species) is clearly visible. Additionally, relaxation of supercoiled DNA substrate (form I pRYG DNA) was inhibited in all reactions that contained VM26. The kit contains marker DNAs, VM-26 control inhibitor, all buffers and complete instructions on using the reagents to screen for inhibitors of topoisomerase II. The manual specifies conditions for gel electrophoresis to resolve all DNA forms; publication quality gels will result.

**Cleavage reactions require more topo II than relaxation assays since the former is a stoichiometric reaction of enzyme. For example, the above reactions contained 4 units of enzyme (units defined by decatenation activity).