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QUICK START GUIDE TO TESTING FOR TOP2 For Cleavage ACTIVITY (Top2 IFP or Interfacial Poisoning)

**VALIDATION EXPERIMENT
Done before testing
Unknown compounds
For Top2 IFP**

We strongly urge all end users test and validate that you are able to detect cleavage complexes with etoposide and topoisomerase II as a control reconstruction experiment. We strongly recommend that you get this experiment to work BEFORE you begin testing unknowns. The following is a plasmid-based assay. Failure to this reconstruction & control experiment may result in wasted reagents!

Rationale: Some drugs, like VP16 are what we call Interfacial Poisons or IFPs. An IFP is a drug that interferes with the concerted breakage/re-ligation reaction with topoisomerases. Essentially, the IFP blocks the re-ligation or resealing step leading to broken intermediates. In the case topo II and VP16, for example, you should see a linear cleavage product but ONLY in the presence of a functional IFP like VP16 (and there are others, see PubMed). When you begin to test unknowns, it is vital that you include a VP16 positive control to show that you can indeed detect the linear cleavage product. This is especially important because it usually takes a relatively high input of enzyme to see the cleavages in vitro. In a cleavage reaction, you need much higher enzyme inputs since the enzyme is 'consumed' by each cleavage event. For this reason, we recommend using at least 4 units of enzyme to see cleavages with Top2. (In contrast, a catalytic reaction is far more efficient since the enzyme is not being 'consumed' but is recycling in the catalytic cycle).

Standard Procedure (see kit protocol, refer to Fig. 1 data below):

Set up duplicate reactions with 0, 0.5 and 1 ul and 2 ul of undiluted enzyme (ie, titrate the undiluted topo II). One set gets **no** VP16 and the other set gets VP16 (we use a relatively high input to ensure that we get a positive result: for example a 1mM to 5mM stock will be diluted 1:10 in the reaction mix to give a final a concentration of 100uM to 500uM).

Typical reaction set up (20 ul final reaction vol, but adjust as needed)

- 5x A+B buffer 4 ul
- Plasmid DNA 1ul (or 100-150 ng input)
- VP16 Drug (5mM) 2ul (or 0ul; compensate with 2ul of water)
- Topo II Variable (usually 4 units minimum)
- D²H₂O Bring to 20ul TOTAL VOLUME with water

Incubate for 30' @ 37°C.

Terminate reactions per protocol (1%SDS, Proteinase K, CIA Extract; you must digest with PK)

Add loading dye (blue juice) per protocol **load half** of the sample onto a 1% EB gel along with relevant markers (relaxed, linear DNA). We typically run TWO gels: one EB and one non EB.

EB gels have 0.5ug EB/ml in gel and buffer

- Run the gel at sufficient voltage (100v is a good start) to move the dye front about 75% down gel in about a 1-1.5h time frame; destain water 10' with a few changes of the water and photograph.
- DO NOT let gels sit around too long before imaging

Non-EB gels: Run gel then stain with 0.5ug/ml EB after electrophoresis. Run as above.

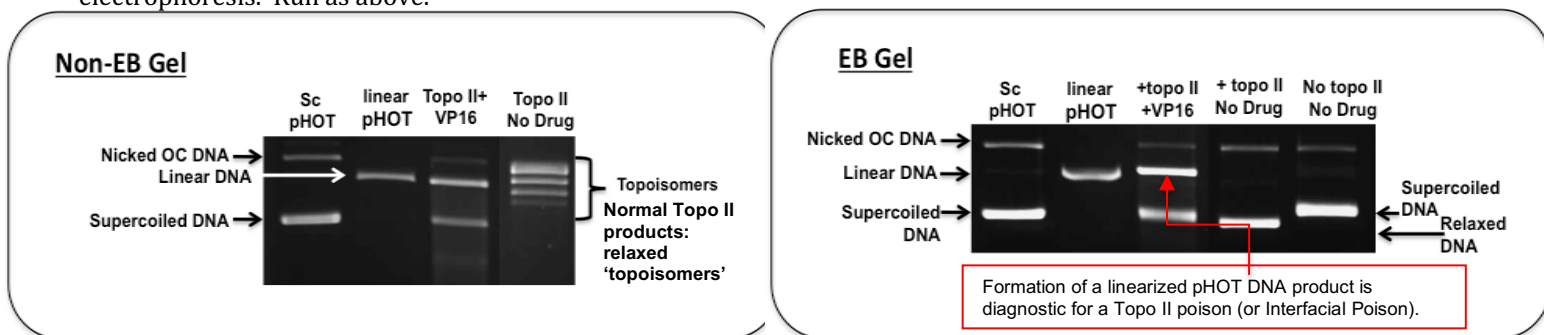


Fig. 1 Topo II Reaction Products Resolved on Non-EB and EB Gels.

Topo II reactions were carried out in a final volume of 20 ul (see above protocol). Reactions were terminated with 1% SDS, digested with proteinase K and extracted with CIA. The final volume after addition of Loading Dye was approximately 26 ul. Two agarose gels (1%) were prepared using a minigel unit. One was cast and run in the absence of EB and the other with 0.5 ug EB/ml (and electrophoresed in a gel containing 0.5 ug EB/ml). Gels were run at 50v for 45-50 min and either stained with EB (non-EB gel) or destained with water (EB gel) per protocols given above. The data show the positions of nicked open circular (OC) DNA which is pHOT1 DNA containing at least one single stranded nick. Topoisomers are relaxed DNA forms that resolve after incubating with topo II in the absence of any drugs; these topoisomers are fully circular and contain no single stranded interruptions. These topoisomers are diagnostic for strong topo II catalytic activity and demonstrate that the enzyme is showing excellent activity. To see this result, a non-EB gel must be used in the analysis. The EB gel (lower) is ideal for detecting topo II cleavage products, including linear DNA (see VP16 reaction where a prominent linear band can be seen). Note that supercoiled DNA substrate and relaxed DNA products are rather poorly resolved in EB gels. In some cases, it is very difficult to demonstrate topo II catalytic activity in this gel system as a result (depending on conditions of electrophoresis).