QUICK START GUIDE TO TESTING FOR TOPOISOMERASE II BY PLASMID RELAXATION.

VALIDATION EXPERIMENT
Done before testing
Unknown compounds
For Catalytic inhibition
Or cleavage (poisoning)
By Top2a.

WHEN YOU FIRST RECEIVE A SHIPMENT OF TOP2, WE STRONGLY RECOMMEND PERFORMING A SIMPLE TITRATION EXPERIMENT BEFORE YOU START TESTING OF UNKNOWNS.

1. FIRST, When you receive your product, store as directed by the product literature. Often this means placing immediately at -70°C. In some cases you can thaw and aliquot the enzyme (not less than 5 ul / tube) to avoid repeated thaw-freeze cycles (which is hard on the enzyme).
2. SECOND: Set up a titration control experiment designed to test activity in your hands, using your equipment and your lab materials. The purpose of this simple titration is to establish conditions for separation/resolution of substrate and products. It also demonstrates the marker locations relative to reaction products.

PROCEDURE:

Set up 4 reactions with 0, 0.5 and 1 ul of undiluted enzyme (optionally do 0, 0.5, 1.0, 1.5, 2.0 ul).
When you assemble the reactions, it is vital that you mix the reactants in the correct order:
Water first > buffer (ie A+B premixed then added for topo II enzymes) > DNA > Enzyme last. ASSEMBLE ON ICE.
Move to temperature block or water bath, Incubate for 30' @ 37°C.
Terminate reactions per protocol
Add loading dye
Load a 1% gel along with relevant markers.

This is a catalytic reaction and you should NOT include Eth.Bro. in gel or running buffer.

- Run a NON-Ethidium bromide gel (no EB in gel or buffer)
- Try to run gels NO LONGER than 2 h. (sufficient voltage to get blue dye about 50-75% down the gel).
- Stain with EB for 10-15', destain water 10' with a few changes
- DO NOT let gels sit before imaging (to keep bands tight)

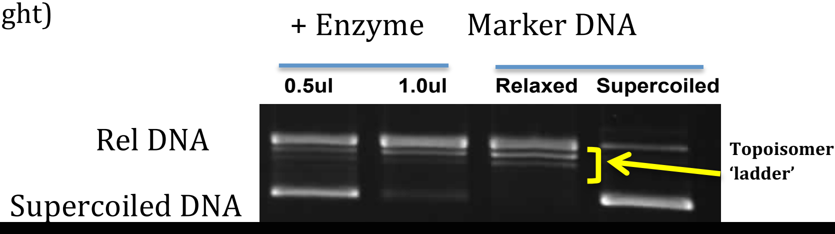

Fig 1. Topo II Titration data. Your data should look similar to this. This is a non-EB gel showing supercoiled and relaxed DNA markers. At 0.5ul of topo II, there is about 50% activity and 1 ul is >80% activity. This is an expected result. If you use too little enzyme, you may see no activity. For this reason, we recommend titrating input topo II over a range.

HINTS:

1. ALWAYS add enzyme last or it will deactivate!
2. ALWAYS run gel markers.
3. ALWAYS test multiple input concentrations of enzyme.
4. For drug testing this experiment must work.. otherwise you are wasting reagents by not doing p
 You must see good activity at this stage!
6. When inspecting gels: look carefully for topois when you see a topoisomer ladder, even if weak, it working as it should.
7. If you wish to test relaxation activity always ru

Topo enzymes are labile and sensitive to freezing this by aliquoting your enzyme; also, keep the thav when working with it on your bench.

For detailed background on application and use of
https://www.youtube.com/watch?v=B4s

It is important to note that our enzyme is sold using KDNA units.

Since the K DNA is a preferred DNA substrate (over plasmid) it will typically show greater activity using this DNA substrate (as opposed to a plasmid DNA substrate) and you may need to run twice as much enzyme to relax plasmid DNA compared to that required to decatenate a KDNA substrate.

This is especially noticeable when detecting cleavage activity by Top2 using plasmids. Typically, we recommend running at least 4 units of enzyme to see cleavages (linear DNA) with a good top2 poison such as VP16.