Research, Diagnostic and Therapeutic
Biomedical Reagents, Kits and Services
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QUICK START GUIDE TO TESTING FOR TOP1 For Cleavage ACTIVITY (Top1 poisoning)

VALIDATION EXPERIMENT Done before testing Unknown compounds For Catalytic inhibition Or cleavage (poisoning) By Top1.

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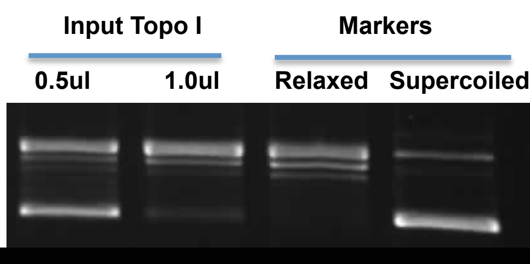
If experience low or no activity OR NO CPT CLEAVAGES with our enzymes, consider doing a 'reconstruction' titration experiment as follows. Always remember this: CPT is technically NOT an inhibitor! It is a Top1 poison: meaning that it induces cleavage of DNA. Also note that detecting cleavages with Top1+CPT usually requires MORE enzyme than relaxation, so you will need excess Top1 in such cleavage reactions. Similar principles apply to Top2 and etoposide. To ensure success with your screening assays for top1 (or top2) poisoning drugs (aka interfacial poisons or IFP), pls do the following.

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 (applies to Top1):
Set up reactions with 0, 0.5 and 1 and 2 ul undiluted enzyme without CPT and the same reactions with CPT (use 20-50uM). NOTE: Avoid DMSO concentrations more than 0.5% as this will inhibit activity (keep DMSO as low as possible). You can dilute the DMSO in aqueous (TE buffer works fine). For example, a 10x stock of CPT (for dilution into your final reaction mix) should be less than 5% maximum (giving 0.5% final in the reaction). The negative control should have equivalent amounts of DMSO.
When you assemble the reactions, it is vital that you mix the reactants in the correct order:
Water first > buffer > DNA > Enzyme> always add drug (or CPT control lane) last. ASSEMBLE ON ICE.
Move to temperature block or water bath, Incubate for 30' @ 37°C.
Terminate reactions per protocol (usually SDS and PK digest)
Add loading dye
Load a 1% gel along with relevant markers. We recommend a CIA extraction before loading to improve gel quality.

For PLASMID BASED ASSAYS:
- Run **both** a NON-Ethidium bromide gel and an EB gel m(0.5ug/ml in and gel buffer).
- Divide reactions in half on one and half on the other gel.
- Be sure you DO NOT contaminate the non-EB gel with EB!!! Be sure to rinse all gel units thoroughly.
- Try to run gels NO LONGER than 2 h. (sufficient voltage to get blue dye about 50-75% down the gel).
- for NON-EB: Stain with EB for 10-15', destain water 10' with a few changes
- for EB: destain with water as above.
- DO NOT let gels sit around too long before imaging.

Figures 1 and 2 below show bands in non-EB and EB gels, respectively. For cleavages, you MUST run an EB gel and quantify formation of the nicked DNA species. NOTE THAT SUPERCOILED AND RELAXED DNA DO NOT SEPARATE WELL IN EB GELS.



Nicked DNA (Form II DNA) is the TOP1 cleavage product that should be quantified to determine the efficacy of a Top1 poison (red circle).