TopoGEN

Products and Services for Cancer Research.

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A Full Service Contract Research Organization For Mechanism Based Anti-cancer / Anti-infective Drug Discovery

Do you need a highly detailed, robust assessment of a potential topoisomerase targeting by a novel drug, compound or natural product? We can help!

With over 20 years as the trusted CRO for topoisomerase research, TopoGEN delivers clear, publication-quality data — fast. We combine rigorous assays (using only pure topo-enzymes, never extracts) with strategic insights to help you unlock your compound's mechanism of action. Our pricing is tailored to your project, complete with positive and negative controls at no extra cost, and we stand by our results with full confidentiality and data ownership. From assay design to in-depth reporting, we're your partner in driving meaningful, publishable discoveries — efficiently and cost-effectively.

- **Trusted Expertise:** 20+ years as the go-to CRO for topoisomerase targeting, with unmatched in vitro & in vivo experience.
- **Publication-Quality Results:** Clear, cosmetic data with rigorous positive & negative controls — guaranteed results you can publish.
- **Cost-Effective & Custom:** Competitive pricing tailored to your exact project needs, with follow-up services offered at a discount.
- **Strategic Support:** Beyond testing — we help you design, analyze, and plan next steps, finishing with a comprehensive data package that's entirely yours.
- **Rapid Turnaround & Total Confidentiality:** Efficient timelines, full data ownership, and NDAs to protect your IP.

Clear Cut Results

Our data will provide value for planning the next phase of R&D with your specific test agent(s). We guarantee clear cut results. We will repeat it, at our expense, to ensure this.

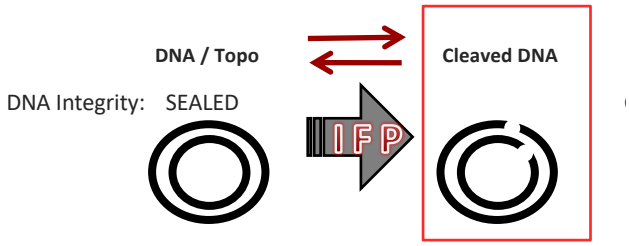
Let us save you time and labor by using a multi-tier approach to testing. Our methods will quickly assess potential targeting. If the results are clearly negative, there is no need for follow-up. If we obtain a hit, we will work closely with you to design next logical steps to reveal mechanistic details (IFP or CIC, see inset below). We will also offer follow-up studies at a discount. In all cases if the results are not unambiguous, we repeat the analysis, without charge, until it is crystal clear.

Useful background information on Topo Targeting Agents.

Topoisomerases are well-established and effective anti-cancer drug targets as well as anti-infective (antibiotic) targets. If you have a natural product, synthetic or semi-synthetic agent and it intersects with a topoisomerase pathway (either a topo-enzyme itself or along the topo axis) there is an excellent chance that your novel compound will work clinically. Of course there are caveats, since in vivo events and unique pharmaco-dynamics may impact on clinical efficacy. Nonetheless, as a first approximation, demonstrating a specific interaction with a topo enzyme is extremely valuable information about a chemical space.

Topo targeting agents operate through two well- established mechanisms. These two mechanisms are often distinct modes but may also share commonalities.

FIRST. A highly specific "**Interfacial Poison" or IFP** is a type of inhibition that operates as the topo is engaged in concerted breakage and resealing activity on DNA. The key here is that the cleavage-re-ligation process common to all topoisomerases should be viewed as an equilibrium between a cleavage state and a resealed (re-ligated) state. In its simplest form:



The topo enzyme binds DNA in a search mode (largely through ionic, electrostatic or hydrogen bonding; see left side of mass action equilibrium). In other words, it is not a covalent interaction. Once the enzyme lands on a favorable cleavage site, the cleavage event ensues (red box) which is immediately followed by a topological interconversion (or Linking Number adjustment). It is important to note that upon cleavage, a covalent bond forms between the DNA substrate and topo molecule. The cleavage intermediate is extremely short lived and rapid resealing as a concerted step is essential to retain DNA integrity. An IFP will shift the equilibrium to the right (large arrow), thereby favoring the cleavage complex wherein a covalent bond forms between the DNA substrate and topo molecule. The cleavage intermediate is extremely short lived and rapid resealing as a concerted step is essential to retain DNA integrity. A prototypical IFP will shift the equilibrium to the right (large arrow), thereby favoring the cleaved DNA intermediate.

SECOND. Certain drugs may be classified as **CIC** (or Catalytic Inhibitory Compounds). These agents are generally less specific and simply block activity of the topo enzyme. ATPase inhibitors are CICs as are some intercalators.

TopoGEN staff will work closely with you or your staff to formulate the best possible experimental screen to identify putative IFPs or CICs or both. Upon completion of a project, we will continue to provide service and consulting advice and project input.

Our relationship does not end when the project report is delivered. In all cases, we will work with the client to craft a logical 'what next' strategy. And, because we design our screens to reveal the subtle mechanism of action of any hit, a few key experiments are often all that is required to understand the MOA or mechanism of action. Specifically, assays are designed to reveal catalytic inhibitors and interfacial poisons (see "Useful Background" inset, page 3) within the same set of reactions. This translates to high cost efficiency.

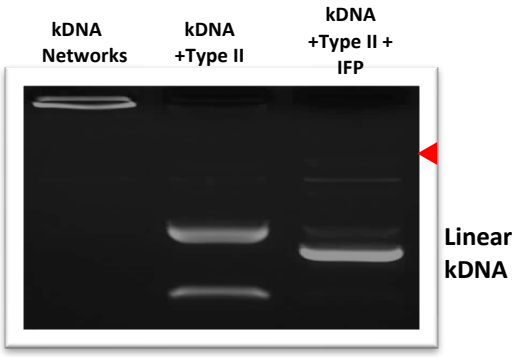
Because we cross-reference our findings with well known topo drugs (as positive controls), we obtain highly significant mechanistic details on drug action.

To ensure scientific rigor, we take steps to prove mutual consistency within each assay. For example as internal proof, we typically QC test topo II with both CPT and VP16 to show that IFP is specific to the former.

Typically, topoisomerases are incapable of introducing permanent DNA breaks unless an IFP is present. There are some exceptions however. If assays are carried out with extremely high ratios of topo to DNA, one can observe some DNA breaks in the absence of IFPs. Because of our experience and know-how, we design our screens specifically to avoid this potential complication. IFP independent cleavages can be detected with extraordinarily high inputs of human topoisomerase I protein, for example. Similarly, E. coli DNA gyrase, when tested in stoichiometric excess, may induce DS DNA breaks in the absence of a known IFP (such as a fluoroquinolone like Ciprofloxacin).

In many (or most) cases, we know your compound will be solubilized in a universal solvent (such as DMSO). High DMSO inputs can be dangerous since false hits may result. This can never happen in our screens for two reasons: First, we establish the operational limits of DMSO inputs for each batch of enzyme; Second, we include controls to rule out a solvent effect.

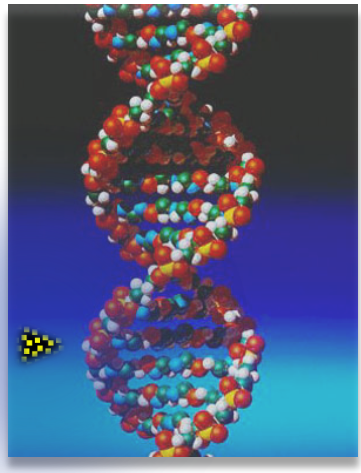
Cost/Benefit Analysis.
Suppose that you are faced with a decision: should you do a single compound screen in-house or with us? While it is hard to generalize due to labor costs and other intangibles that vary widely around the globe, we can provide a rough comparison.
A Single Compound Screen with Top1 or 2a.
We estimate that an in-house screen for Top1 or Top2 with one compound would cost you (in materials, supplies, labor) > $4000. This figure includes your materials and supplies, your labor, multiple screening kits, control drugs, relevant markers, enzymes, dry ice shipping of labile products, ambient temperature shipping of kits, and any tariffs (to name a few). This also assumes that everything will work perfectly the first time AND that your staff will obtain cosmetic data to meet the high standards of evidence required by journal peer review or UPTO. It is our experience that the typical end user will spend considerable extra labor getting the assays established. This inflates your cost with no guarantee of a successful outcome. TopoGEN certifies a clear, publication quality work product for roughly half the cost and a fraction of the time. You will receive well-documented data from professional experts who do this routinely. Our Screening Program has proven very cost effective and will advance your science rapidly and efficiently while stretching grant dollars. We can complete this study for a fraction of the cost with guaranteed results in a fraction of the time (usually a few business days).



Performing your own 'in house' screening with our specialty products is possible but not always practical. Screening one drug with two topo targets will cost >$4000 with no guarantee of publication quality work product. A large part of this cost is the learning curve.

Our Screening program provides a rapid turn around service that is cost effective and flexible!

If you are developing new drugs on a regular basis, enquire about **block screening**. You can secure best prices for a pre-set # of test drugs over a time frame that fits your schedule. We will coordinate with your chemists or staff to test novel compounds as they are made. In this way, we offer the advantages of bulk screening contract pricing (multiple compounds), and flexibility over timing and control of testing. This will match your schedule and make for a cost-effective project.



Real time data.

When you outsource your drug screening to TopoGEN, we will consult with you initially to design the best experimental inroad for the project. If you are interested in a specific target, we will enquire about your need to identify an IFP, a CIC or both. We will provide you with real world data so you can readily visualize the sort of work product that will result. We will also give you realistic turn around times in 'business days' required to complete the study. Shown in Fig. 1 is an actual screen for topo I targeting against an unknown Test Drug A or B. In this screen, we clearly demonstrate that Drug B is a novel topo I IFP drug.

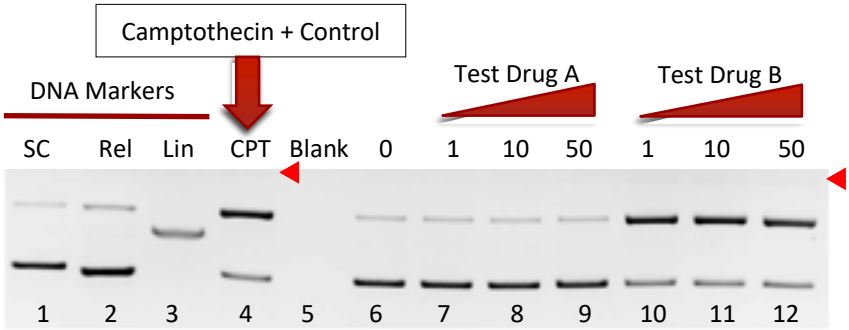


Figure 1. Topo I drug screen.
A plasmid based relaxation assay was performed using supercoiled (SC) pHOT1 DNA (a proprietary substrate designed for topo I screening). Reactions were carried out in the presence or absence of increasing levels of test drugs A and B (uM inputs shown above each lane). Reaction products and markers were loaded onto an agarose gel containing ethidium bromide. This gel system cleanly resolves the topo I cleavage intermediate (nicked open circular DNA, red triangle). This cleavage intermediate is only detected in the presence of an IFP or interfacial poison. The positive control with Camptothecin (10 uM) a known topo I IFP is shown to accumulate the cleavage product (compare lanes 4 and 6 which contain CPT or no drug, respectively). Test drug A had no influence on the relaxation of pHOT1 DNA (lanes 7-9 compared to '0' drug control, lane 6). In contrast, drug B is clearly a topo I IFP as attested by the appearance of nicked open circular pHOT1 (red triangle). These cleavage products are clearly drug dependent. Note that the Campothecin positive control (lane 4) demonstrates that the assay is working as expected. This gives you total confidence that a negative result (lanes 7-9) can be trusted.

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