TopoGEN
Products and Services for **Cancer Research.**

In Vivo Link Kit for Analysis of Topo I or II Covalent Complex Formation Inside Cells (Cat No. TG1021 and TG1022, see also ICE Assay Kit TG1020)

Kit Description

TopoGEN has extensive experience with assays for topoisomerase inhibition in vivo. These experiments allow the investigator to ascertain whether a novel agent is active against endogenous topo in a chromosomal setting in nuclei. An important benefit to this analysis is that one can use any tumor cell or tissue in order to establish clinical efficacy of a test drug against a specific tumor cell line. We use essentially the same basic approach for Topo I as for Topo II. The methods are based upon physically separating the topo/DNA adducts from free DNA and using antibodies to measure bound topo I or II. In this analysis, tissue culture cells are treated with a test compound along with negative controls (no drug) and positive controls (with known inhibitors). The cells are drug treated and rapidly lysed with Sarkosyl which traps some fraction of the endogenous topo on DNA in a covalent cleavage complex. Following detergent lysis, the lysate is diluted to fully dissociate non-covalent DNA/protein complexes. Covalent topo/DNA complexes are resolved on a step CsCl gradient (ionic DNA/protein interactions are also prevented by 5 M CsCl). The gradient resolves DNA, chromatin aggregates, and protein, respectively. Gradients are centrifuged overnight and fractionated. The amount of topo coincident with the DNA peak is a measure of covalent DNA/topo complexes. Topo concentration in the DNA peak is determined by immunoblotting using antibody to topo I or II as probe. In the absence of agents that stabilize the cleavable complex (etoposide, camptothecin) only low levels of topo are found in the DNA peak; this is particularly obvious with topo II since the type II enzyme is not trapped by this method unless an inhibitor is used. In contrast, topo I is trapped to a low extent even in the absence of camptothecin. The ratio of topo at the DNA density and the protein density reflects the relative efficiency of stabilization of the cleavable complexes.

References

Ebert et al., J. Virology 64: 4069-4066, 1990.

Muller and Mehta, Mol. Cell. Biol. 8: 3661-3669, 1988.

Trask and Muller, Proc. Natl. Acad. Sci. 85:1417-1421.

Kit Contents

Camptothecin: supplied lyophilized with Topo I Kits (TG1021)

Etoposide: supplied lyophilized in Topo II Kits (TG1022)

Sarkosyl (20%)

Antibody to Topo I: supplied with Topo I Kits (TG1021)

Antibody to Topo II: 170 kDa form, supplied with Topo II Kits (TG1022)

CsCl Stock Solution

Detailed Instruction Manual

General Procedures

An outline of the method is shown in Fig. 1. Cells to be tested may be a particular cell line, virus infected cell or tumor tissue. Cells are incubated under conditions that favor endogenous topo activity (defined as physiological conditions conducive to cell growth); thus, the endogenous enzyme is engaging the DNA template in a series of breaking and resealing steps. Concurrently, central genetic processes such as DNA replication, transcription and repair are ongoing. The cells are then rapidly lysed with a detergent (sarkosyl). It is important that lysis be carried out while maintaining the cells at 37 C; if the cells are cooled or manipulated prior to lysis, the cleavage complexes tend to re-ligate and yield negative results (see Trask and Muller, 1988). The next step requires purification of DNA away from free protein; however, organic extractions or proteinase digestions must be avoided. We use a step CsCl gradient for this purpose.

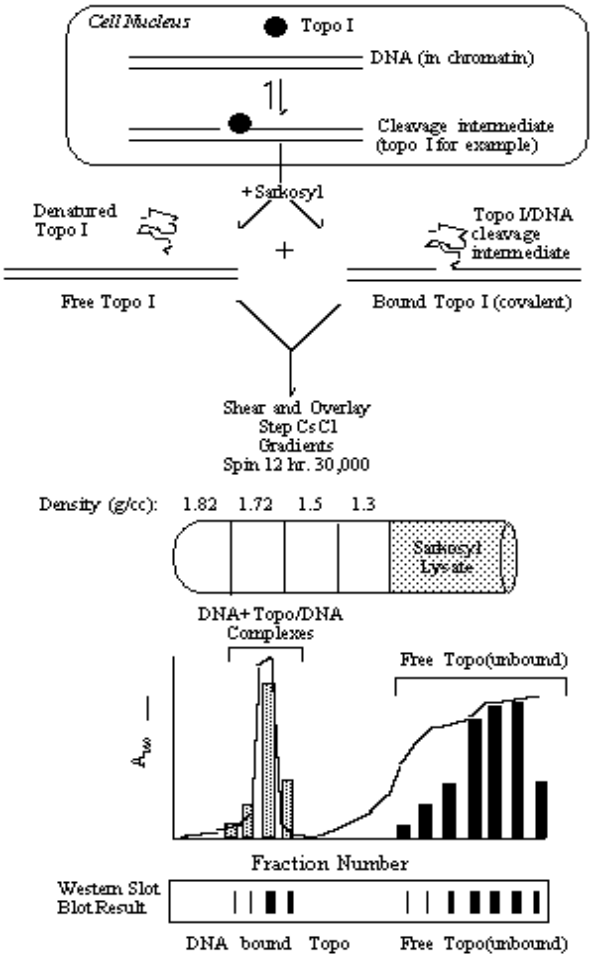

Figure 1. Outline of the In Vivo Link-Kit Protocol.

The density steps are designed to resolve DNA from free protein and there is quantitative recovery of both. Covalent complexes containing topo and DNA sediment to the position of DNA. It is known that covalently bound protein can shift the density of DNA in CsCl and the magnitude of the density shift is proportional to the total amount of protein. In vitro, topo I may produce a density shift of DNA under conditions of stoichiometric excess of protein (data not shown); however, in vivo significantly less protein is coupled to DNA. In fact, topoisomerase does not cause any density shift of genomic DNA in this analysis (Fig. 1) for two reasons. First, the number of bound topo mecules per DNA molecule is very low and in these gradients, complexes behave like free DNA (vs. DNA/protein adducts). Second, the gradients are very steep and it is not possible to resolve small density differences anyway. After CsCl centrifugation, the gradients are fractionated and amount of bound and free topo is measured by Western blotting using slot or dot blots and antibodies developed by TopoGEN. The ratio of bound/free is a direct measure of the cleavable complex formation in the particular cell system. We have found that topoisomerases are difficult (if not impossible) to trap as cleavable complexes within cells in the absence of inhibitors; thus, any test compound that results in detection of topo I or II in the DNA peak (Fig. 1) is most likely a topo-active agent. The In Vivo Link-Kit then allows the investigator to evaluate a compound for activity against topo I and II in different tissue settings or with virus infected cells. Finally, it is possible to combine the analysis of topo I and II in t same experiment. In this case, one can evaluate topo I inhibition using the topo I antibody (Cat# 2012) or topo II using that antibody (Cat# 2011-1). Some drugs may conceivably act upon both enzymes (Trask and Muller, 1988).

References

1. Ebert et al., J. Virology 64: 4069-4066, 1990

2. Muller and Mehta, Mol. Cell. Biol. 8: 3661-3669, 1988

3. Trask and Muller, Proc. Natl. Acad. Sci. 85:1417-1421

4. Drlica and Franco. 1988. Biochemistry 27:2253-2259.

5. Liu, 1989. Ann. Rev. Biochem. 58:351-375.

6. Osheroff, N. 1989. Pharmacol. Ther. 41:223-241.

7. Wang, 1991. J. Biol. Chem. 266:6659-6662.

8. Wang, J.C. 1985. Annu. Rev. Biochem. 54:665-697.