

ICE ASSAY KIT CATALOG TG1020-1REF

QC Data Sticker

Sufficient for 10 Slots to demonstrate ICE Δ



Overview:

TopoGEN has extensive experience with cell-based screens for topoisomerase targeting. Our staff scientists developed and published a method for detecting covalent topo/genomic DNA complexes in live cells (1,2). The method, called the ICE Assay (In vivo Complex of Enzyme) originally required a CsCl gradient to purify genomic DNA and specific topo antibodies to detect trapped topo protein in the DNA fraction. This method, while powerful, is slow, labor intense and requires large expensive equipment. **We have modified the technique to allow direct isolation of DNA/topo complexes in a single step, omitting the CsCl gradient step and rapidly increasing the throughput of the method.** The key to specificity is the use of TopoGEN's highly directed antibody reagents to detect complexes. The ICE Assay can be carried out in a 1-2 days. The analysis can be conducted on virtually any cell or tissue system as long as proper controls are performed and mono-specific antibody reagents are used. Finally, the assay is no longer limited to 6 samples per run (for the ultracentrifuge rotor), so larger throughput is possible. Our new ICE method is rapid and convenient and can be used with any topoisomerase target (I, II, III). Each kit is designed for a specific target (see labeling above). As a companion control/reference, we offer convenient internal controls to efficiently assess and validate the results. A key control is ICE-prepped DNA from Hela cells treated with or without Camptothecin to demonstrate that the assay is working properly. We refer to this as 1020-1REF which is genomic DNA purified per the ICE protocol described in the parent kit #1020-1.

1. Subramanian, D., Furbee, C. and Muller, M. (2000) ICT Bioassay: Isolation of In Vivo Complexes of Enzyme to DNA. DNA Topoisomerase Protocols Vol. II: Enzymology and Drugs. Humana Press.

2. Subramanian, D., Kraut, E., Staubus, A., Young, D., and Muller, M.T. (1995) Analysis of Topoisomerase I DNA Complexes in Patients Administered Topotecan. Cancer Research 55:2097-2103.

Shipping and Storage of Reagents

These products may be shipped ambient, wet ice packs or dry ice. Upon receipt you should store this kit at -20° C for long term or keep at 4°C for short term (<7d).

Introduction.

An important parameter for the ICE assay is the ICE-Delta which is the ratio of plus control Drug signal to minus Drug signal control. The ICE Δ ratio should be 2-6 fold or more for a valid result. In other words if the ICE Δ ratio was 1.0, it would indicate that covalent complexes are NOT being detected by the assay. To serve as an internal control, 1020-1REF-NEG is genomic DNA complexes prepared from Hela cells that are non-drug (Camptothecin) treated, and 1020-1REF-POS is genomic Hela DNA from cells treated with CPT for 30' prior to harvest.

Kit Contents are as follows: (Note that Sufficient material is included for 10 individual Western Blots.

1020-1REF-NEG: Complexes (DNA-protein) prepared from non-drug treated Hela cells.

Genomic DNA concentration = 2.5ug/ul (10ul provided)

1020-1REF-POS: Complexes from Camptothecin Treated Hela cells.

Genomic DNA concentration = 2.5ug/ul (10ul provided)

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Using the marker set

1. The provided markers (-/+ camptothecin treated HeLa Cells) can be used to demonstrate on Westerns slot or dot blots the ICE Δ parameter defined in the companion kit (1020-1).
2. As shown in Fig. 1, this has been reconstructed and serves as an effective positive control.
3. The data show that slot-blotting from 0.75 ug to 3.0 ug per slot is sufficient for this purpose.
4. For each marker:
 - 1020-1REF-NEG: comes from non-drug treated Hela cells (clear background)
 - 1020-1REF-POS: comes from CPT treated Hela cells (strong signals over range tested)

We recommend testing over the demonstrated range (0.75 to 3.0ug/slot) or at a single concentration of 2.5ug (1ul) of the stock genomic DNA provided. As shown in Fig. 1, you should be able to use as little as 1ug.

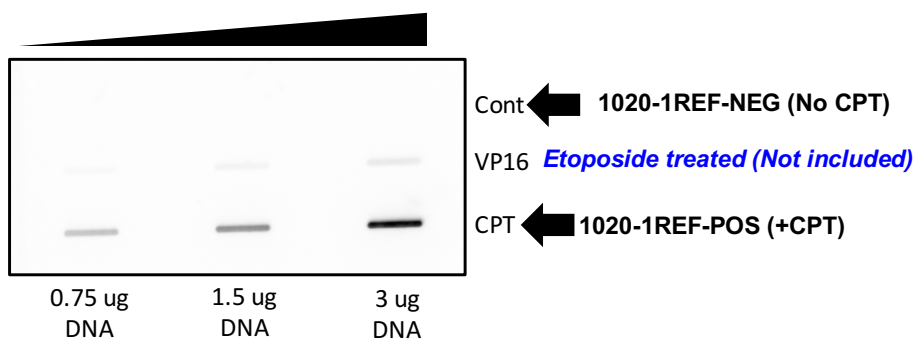


Fig 1. ICE Blot Data using markers. The indicated amounts of each reference genomic DNA was spotted onto the membrane, which was probed with a monoclonal anti-HuTop1 Antibody (1:2500) and subsequently processed using the protocol in the companion Kit product (TG1020-1). For specificity demonstration, we treated HeLa cells with a Top2 poison (Etoposide) and harvested genomic DNA and placed it on the blot (middle, labeled in **Blue**). The weak signal represents Top1 recruitment to sites of DNA damage; however it is specific for Top1. This VP16 treated DNA is not included in the reference marker set.

FAQ: 1020-1REF

Should I use 3ug of the marker; this seems like a lot? Not necessarily, you can conserve on things by using less. As little as 0.5ug may work but your detection reagent system may or may not allow such low inputs. We recommend starting initially with 3ug to establish things first however.

Should I titrate multiple inputs? It makes for a nice cosmetic image readout but is not essential... a single input concentration may work as well.

You show data with VP16 in Fig 1 yet did not provide this reagent... why is this? The VP16 treated Hela cell DNA is provided to demonstrate specificity of the AB probe and is not necessary for use as a control. The purpose of the REF DNAs provided is to demonstrate that the blotting is working and to show optimal ICE Delta data. If you wish, this control can be added for a 25% upcharge (please contact support@topogen.com).

Would you recommend testing these reference controls first, before doing any experiments with my own cells? It would certainly be a good idea. In this way you will clearly demonstrate that your western blotting methods are working as expected. This is a conservative way to proceed and will save on reagents.

How long are these REFs good for...ie, what is the shelf life? The covalent Top1/DNA adducts are very stable and should be around for at least 6 months if stored properly (-20oC).

Why is it important to show a clean ICE Δ ? It is very crucial. You need to show that in the absence of Camptothecin, there is little or very low signals. Otherwise, you may not have reliable data.

Can we use these REFs and NOT run a minus drug control? Absolutely NOT. These REFs are designed to validate your blotting technique. You must always run minus and plus drug controls with CPT (or other Top1 poisons) in your experimental analysis. In other words, you must generate an internal ICE Δ for each experiment.