User Manual

Human Topoisomerase II Immunofluorescence Kit

Catalog Number TG1030

Overview:

This kit contains reagents for cytolocalizing human topoisomerase II (topo IIa or p170) in tissue culture cells. Purified topoisomerases and antibodies are available from TopoGEN and may be ordered on line at www.topogen.com .

Shipping and Storage of Reagents

The kit may be shipped at ambient temperature or on ice (dry ice or wet ice). Avoid frequent freeze/thaw cycles.

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I. Introduction

A. Summary

This assay kit contains all the reagents for cytolocalization of human, p170 topo II using immunofluorescence. The system is opitimized for use with FITC labeled secondary antibody with adherent cells. The method may work with other cells besides humans. Mouse, rat and monkey cells will cross react with the human topo II antibody (specific for p170 or topo IIα); however the end user should perform an optimization experiment for their cells or system.

B. Kit Contents

Anti-topo II (p 170) Antibody (TG2011-1). This is a rabbit antibody (polyclonal) directed against the C-Terminal region of p170. A peptide was used to raise this antiserum; the antibody has been immunoaffinity purified and is ready to use. Store at -20°C.

Peptide reagent: (TG2011-2). A total of 50 ug of lyophilized peptide is included. The seqeunce is the C- terminal 16 amino acid residues in p170. This peptide should be rehydrated with 25 ul of sterile water to give a working stock of 2 ug/ul Store at -20°C.

C. Protocol

This protocol was developed for use with adherent tissue culture cells (HeLa, Vero, 3T3, etc.). Applications involving suspension cell culture, thin sections or horseradish peroxidase staining will require some modifications worked out by the investigator. It is important to note that because topo II is highly enriched in chromosomes, an internal control is built into the method; one should see brightly fluorescent chromosomes (in mitotic cells of course).

1. HeLa cells are grown on small cover slips in tissue culture plates to approximately 40% confluence (cells should be actively growing since topo II is cell cycle regulated).

2. Remove media and rinse cover slips gently (two times) with lx D-PBS (8.06 mM Na2HPO4.7H20, 1.47 MM KH2PO4, pH 7.1, 137 mM NaCl, 0.27 mM KCl, 0.68 mM CaCI2, 0.49 mM MgC12).

3. Fix cells at room temperature with 3.7% Formaldehyde in D-PBS for 10 min.

4. Wash cells for 5 min with D-PBS. Repeat 5 min wash.

5. Wash with 0.1% SDS and 1% 2-mercaptoethanol in D-PBS for 10 min at room temp.

6. Rinse briefly with D-PBS and permeabilize with cold (-20°C) methanol for exactly 2 min.

7. Remove methanol and rinse once with D-PBS.

8. Wash three times (5 min per wash) with KB buffer (10 mM Tris-Cl, pH 7.5, 150 MM NaCl, 0.1 % Triton X-100, 0.1% Bovine serum albumin). Leave in KB buffer after last wash.

9. Dilute primary antibody (TG2011-1) 1:10 or 1:100 (1:10 is best but either of these dilutions are acceptable for HeLa cells). We recommend you try several dilutions with your cells. Typically, one should start with concentrated primary AB (1:2) and go down to 1:20.

10. Procedure for incubating with primary antibody:

i) Use scalpel to lift coverslip out of KB wash and remove excess KB carefully by blotting with a tissue or Kimwipe. Hold the cover slip at one edge with tweezers or forceps and mark the top of the tweezer with a dot (use a permanent marker) or with a piece of tape so the cell side up orientation of the cover slip is always known. With the cell-side facing up, apply 20 ul of diluted (1:10 for example) rabbit antibody directed to human topo II (TG2011-1).

ii) Separate the lid from the bottom of a sterile 35 mm petri dish.

iii) Invert the bottom part of the dish onto the cover slip so that the antibody/cell surface is in direct contact with the plastic. Turn the dish back over and now place on the petri dish lid and incubate at 37°C for 2 hrs. (this process eliminates evaporation).

11. Add 1 ml of KB to the petri dish with a sterile scalpel or forceps, flip the coverslip over so that now the cell side is up. Remove the KB and wash the covers twice with about 2 ml of KB (5 min per wash).

12. In the dark, dilute the secondary antibody in KB at 1:100 (goat anti-rabbit tagged with rhodamine or fluorescein). This reagent is not supplied in the kit and is available commercially from a number of different vendors.

13.Use scalpel to lift the coverslip up and grab the edge with forceps. Blot off excess KB as (Step10) and hold the coverslip cell side up. Add 35 ul of secondary antibody. Take a fresh 35 mm plastic petri dish, invert onto coverslip, turn petri dish over and cover with lid (as described in Step 10). Incubate for 2 hrs. at 37°C.

14. Remove lid from plate, add 1 ml of KB per dish and gently turn coverslip back over (use a scalpel and forceps). Wash with 2 ml KB for 5 min. Remove KB and add back 2 ml of fresh KB per dish. Washing overnight at 4°C will result in a lower background.

15. Remove coverslip from dish, remove excess KB, add 15 ul of mounting medium (50% glycerol, in 10 mM Tris, pH 7.5, 100 mM NaCl, 1 mM EDTA) and mount onto pre-labeled slides for viewing.

D. Controls and Important Considerations

1. As noted above, topo II has a characteristic distribution pattern in cells that clearly indicates whether the procedure is working. In interphase cells, the pattern of distribution of topo II is almost totally nuclear and appears to be punctiform or "pin-point" in nature. The signal is clearly amplified in metaphase cells when chromosomes are visible. There should be about a 5-10 fold increase in fluorescent intensity in the mitotic cells.

2. The peptide that is included is an important control reagent. Incubating the cells with peptide plus primary topo II antibody should neutralize the fluorescent signal.

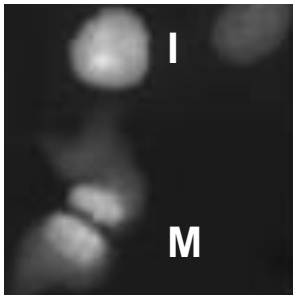

Fig. 1. Low resolution image of human topoisomerase II distribution in mitotic (M) and interphase (I) cells.