

Topoisomerase II Immunofluorescence Kit. Cat # 1030

Kit Description:

This assay kit contains all the reagents for cytolocalization of human, p170 topo II using immunofluorescence. The system is optimized for use with FITC or Rhodamine labeled secondary antibody with adherent cells. The method may work with other cells besides human. Mouse, rat and monkey cells will cross react with topo II antibody. The antibody is specific for p170 (topo II alpha).

Kit Contents:

Anti-topo II (p170) Antibody, Cat# 2011-1. 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. Kit contains 100 ul of antibody. Store at -20oC.

Peptide reagent: Cat# 2011-2. A total of 50 ug (at 2 mg/ml) of peptide included. The sequence is the C-terminal 16 amino acid residues in p170. This peptide is in sodium phosphate buffer and should be stored at -20oC.

Detailed Manual of Operation.

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).

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 latter 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.