ProMab Biotechnologies, Inc.

Protocol

Immunocytochemistry (ICC)

OVERVIEW

Immunocytochemistry (ICC) is classically defined as a procedure to detect antigens in cellular contexts using antibodies. Immunocytochemistry (ICC) is a common laboratory technique that uses antibodies that target specific peptides or protein antigens in the cell via specific epitopes. These bound antibodies can then be detected using several different methods. ICC allows researchers to evaluate whether or not cells in a particular sample express the antigen in question. In cases where an immunopositive signal is found, ICC also allows researchers to determine which sub-cellular compartments are expressing the antigen.

Immunocytochemistry (ICC) is an immunological technique that is very similar to Immunohistochemistry. Immunocytochemistry (ICC) is used to visualize the presence of a specific protein or antigen in cells (cultured cells, cell suspensions), rather than tissues. The types of cell samples that can be investigated include blood smears, cultured cells, cell suspensions, and cytospins. Each type of cell sample is prepared and treated slightly differently, but the fundamental use of primary antibody, secondary antibody and color development is very similar between Immunocytochemistry and Immunohistochemistry.

For immunocytochemistry (ICC), sample preparation involves fixing the target cells to a slide. Cells can be attached to a solid surface by several methods: adherent cells may be grown on microscope slides; cell suspensions can be centrifuged onto glass slides (cytospin),or bound to solid support using chemical linkers.
body.

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To ensure access of the antibody to its antigen, cells must be fixed and permeabilized. In an ideal situation, fixation would immobilize the antigens while retaining native cellular architecture and permitting unhindered access of antibodies to all cells and subcellular compartments.
Fixation methods fall generally into two groups: organic solvents and cross-linking reagents. Organic solvents, such as alcohols and acetone, remove lipids and dehydrate the cells, while precipitating the proteins on the cellular architecture. Cross-linking reagents (such as paraformaldehyde) form intermolecular bridges, normally through free amino groups, thus creating a network of linked antigens. Cross-linkers preserve cell structure better than organic solvents, but may alter the structure of some cell components, so much so that they are not recognized by the primary anti

SPECIMEN PREPARATION

1. Coat coverslips with poly-L-lysine for 5 min at room temperature.
2. Aspirate liquid, allow coverslips to dry completely and sterilize them under UV light for at least 30 min.
3. Grow cells on glass coverslips .Note:If the specimen is suspension cell , cytospin or smear preparation is needed.

IMMUNOSTAINING

1. Rinse coverslips two times with PBS.
2. Fix cells with 4% paraformaldehye in PBS for 15 min at room temperature.

Note: Paraformaldehye is toxic, use only in fume hood.

3. Aspirate fixative, rinse two times in PBS for 5 min each.
4. Permeabilize cells with 0.1-0.5% triton x-100 in PBS for 10 min.

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Note: Permeabilization is only required when the antibody needs access to the inside of the cells to detect the protein. These include intracellular proteins and transmembrane proteins whose epitopes are in the cytoplasmic region.

5. Aspirate triton x-100, rinse two times in PBS for 5 min each.
6. Incubate cells in 10% normal goat serum in PBS for 30 min at room temperature.
7. Aspirate goat serum, incubate sections with primary antibody at appropriate dilution in PBS overnight at 4°C or 1 hour at 37°C.
8. Rinse three times in PBS for 5 min each.
9. Incubate cells with fluorochrome-conjugated secondary antibody at appropriate dilution in PBS for 1 hour at 37℃ in dark.
10. Rinse three times in PBS for 5 min each in dark.
11. Incubate cells with 1 μg/ml DAPI.
12. Mount coverslip with a drop of mounting medium.