Echelon Biosciences Inc.

Protocol for immunocytochemistry (ICC) with protein antibodies

This protocol is recommended as a general guide for immunostaining of cells with antibodies for detecting proteins of interest. While this procedure may be suitable for staining lipids in some systems, we do not recommend it as a starting point. Please see our alternative protocol(s) for ICC with lipid antibodies.

All steps during fixation, washing, and staining should be performed on a rocking or rotating platform to allow for gentle mixing during incubation. Please read the entire protocol before beginning.

Procedure :

1. For cells grown on coverslips, aspirate culture media and wash cells once briefly.
   - Suggested buffers and solutions for this protocol can be found in the table below.
   - TBS will be used as an example for this protocol.
2. Fix cells by adding 4% paraformaldehyde in TBS to cells in media for 10-20 minutes at room temperature (RT).
   - The volume of fixation solution should cover the cells. For a 6-well plate, 500 µl/well should be sufficient.
3. Remove the fixation solution and wash for 5 minutes three times with TBS.
4. Permeabilize the cells with 0.5% Saponin-TBS or 0.1 % Triton X-100-TBS at RT for 10-15 minutes.
   - Permeabilization is only necessary for intracellular proteins. For cell surface proteins, or membrane proteins with external domains or epitopes, this step may be omitted.
5. Remove the permeabilization solution and wash for 5 minutes three times with TBS.
6. Block with 5-10% Serum in TBS for 30 minutes at 37 °C.
   - The choice of serum should be made according to the host spcies of the secondary antibody, i.e. use normal goat serum in the blocking solution if a goat secondary antibody is to be used.
7. Dilute the primary antibody in TBS to the concentration suggested on the technical data sheet. Remove the blocking solution and add the primary antibody solution. For a 6-well plate, 500 µl/well should be sufficient. Incubate for 60 minutes at RT.
8. Remove the primary antibody solution and wash for 5 minutes three times with TBS.
9. Dilute the secondary antibody in blocking solution to the concentration suggested on the technical data sheet. Incubate for 30-60 minutes at RT.
   - For fluorescently conjugated secondary antibodies, this step and all remaining steps until mounting should be performed covered or in the dark. For a 6-well plate, 500 µl/well should be sufficient.
10. Remove the secondary antibody solution and wash for 5 minutes three times with TBS.
11. Following the last wash, remove the coverslips one at a time and wick away the excess wash buffer or allow it to drain off.
    - DAPI or alternative nucleic acid stains may also be applied at this time if they are not present in the mounting media.
    - Excess nucliec acid stain should also be removed prior to mounting.
12. Add the mounting media to a microscope slide and mount and seal the coverslip. Repeat this for all stained samples and store at 4 °C in the dark until imaging.

Suggested Buffers for Optimization

Wash Solutions		Block Solutions		
TBS TBS tablet (Amresco K859) in 100 mL dH2O For **TBS-T** Add 0.1% (v/v) Tween-20	**PBS** Dissolve PBS Tablet (Sigma P4417) in 200 mL H2O For **PBS-T** Add 0.1% (v/v) Tween-20. Adjust pH to 7.2	**TBS or PBS + 3% BSA** Add 3 g fatty acid free BSA (Seracare 1900-0016) to 100 mL TBS or PBS For **PBS-T + 3% BSA** Add 3 g BSA to 100 mL PBS-T	**TBS or PBS + 1% milk** Add 1 g non-fat dry milk to 100 mL TBS or PBS	**TBS or PBS +0.1% ovalbumin** Add 0.1 g ovalbumin (Sigma A5253) to 100 mL TBS or PBS

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