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Protocol for immunocytochemistry (ICC) with lipid antibodies

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

Immunostaining of cellular lipids requires more specific procedures than are provided in general ICC protocols owing to the different cellular compartments in which they are found and how those compartments are affected by fixation and permeabilization reagents. Here, we outline protocols for the staining of lipids in different cellular compartments. Please read the entire protocol before beginning.

Procedure, general lipid staining:

1. Fix cells by adding 4% paraformaldehyde to cells in media for 20 minutes at room temperature.
2. Wash three times with TBS.
3. Permeabilize the cells with 0.5% Saponin or 0.01% Digitonin at RT for 15 minutes.
4. Wash three times with TBS.
5. Block with 10% Goat Serum in TBS either overnight at 4 °C or 30 minutes at 37 °C.
6. Add the anti-lipid or anti-PIP antibody diluted in 10% Goat Serum in TBS to the concentration suggested on the technical data sheet. Incubate for 60 minutes at 37 °C.
7. Remove the primary antibody solution and wash for 5-10 minutes in TBS-Goat Serum 1%. Repeat twice.
8. Dilute the secondary antibody in 10% Goat Serum in TBS to the concentration suggested on the technical data sheet. Add the secondary antibody solution and incubate for 45 minutes.
    - For fluorescently conjugated secondary antibodies, this step and all remaining steps until mounting should be performed covered or in the dark.
9. Remove the secondary antibody solution and wash for 5-10 minutes in TBS. Repeat twice.
10. Remove excess wash buffer by blotting the edge of the coverslip.
    - 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.
11. 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.

Procedure, plasma membrane staining:

1. Remove culture media from cells and fix with 4% formaldehyde (FA) and 0.2% glutaraldehyde (GA) in PBS for 15 minutes at room temperature (RT).
    - Fixation may also be performed at 4 °C for 3 hours.

    Steps 2-8 should be performed on ice or at 4 °C using pre-chilled buffers and solutions.
2. Remove the fixation solution and rinse three times with 50 mM NH4Cl in PBS.
3. Block and permeabilize the cells with Buffer 1 (see recipe below) supplemented with 5% normal goat serum, 0.5% saponin, and 50 mM NH4Cl for 45 minutes.
4. Dilute the primary antibody in Buffer 2 (see recipe below) to the concentration suggested on the technical data sheet. Remove the block and permeabilization solution and add the primary antibody solution. Incubate for 60 minutes.
5. Remove the primary antibody solution and wash for 5-10 minutes in Buffer 1. Repeat once.
6. Dilute the secondary antibody in Buffer 2 to the concentration suggested on the technical data sheet. Add the secondary antibody solution and incubate for 45 minutes.
    - For fluorescently conjugated secondary antibodies, this step and all remaining steps until mounting should be performed covered or in the dark.
7. Rinse the slides or coverslips with Buffer 1 four times.
8. Post fix cells in 2% FA in PBS for 10 minutes.
9. Following post-fixation, bring the cells to RT. All remaining steps may be performed at RT.
10. Rinse cells three times with 50 mM NH4Cl in PBS, then once with MQH2O or dH2O.
11. Remove excess wash buffer by blotting the edge of the coverslip.
    - 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.

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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.

Buffer 1: (20 mM PIPES, pH 6.8, 137 mM NaCl, 2.7 mM KCl)
Buffer 2: (20 mM PIPES, pH 6.8, 137 mM NaCl, 2.7 mM KCl) + 5% normal goat serum, 0.1% saponin

Procedure, Golgi apparatus staining:

All steps should be performed at room temperature.

1. Remove culture media from cells and fix with 2% FA in PBS for 15 minutes.
2. Remove the fixation solution and rinse three times with 50 mM NH4Cl in PBS.
3. Permeabilize the cells with Buffer 1 supplemented with 20 µM digitonin for 5 minutes.
4. Rinse three times with Buffer 1 and block the cells in Buffer 1 supplemented with 5% normal goat serum and 50 mM NH4Cl for 45 minutes.
5. Dilute the primary antibody in Buffer 2 to the concentration suggested on the technical data sheet. Remove the block and permeabilization solution and add the primary antibody solution. Incubate for 60 minutes.
6. Remove the primary antibody solution and wash for 5-10 minutes twice in Buffer 1.
7. Dilute the secondary antibody in Buffer 2 to the concentration suggested on the technical data sheet. Add the secondary antibody solution and incubate for 45 minutes.
   - For fluorescently conjugated secondary antibodies, this step and all remaining steps until mounting should be performed covered or in the dark.
8. Rinse the slides or coverslips with Buffer 1 four times.
9. Post fix cells in 2% FA in PBS for 10 minutes.
10. Rinse cells three times with 50 mM NH4Cl in PBS, then once with MQH2O or dH2O.
11. Remove excess wash buffer and dry cells.
   - 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.

Buffer 1: (20 mM PIPES, pH 6.8, 137 mM NaCl, 2.7 mM KCl)
Buffer 2: Buffer 1 + 5% normal goat serum.

Procedure, nuclear membrane staining:

All steps should be performed at room temperature.
For staining of the nuclear membrane, please refer to and follow the protocol described for 'Golgi apparatus staining' and substitute 0.2% Triton X-100 for digitonin.

Additional Notes

1. These protocols are recommended as starting points and are based on published data for lipid and phosphatidylinositol phosphate immunostaining. Users are encouraged to adjust the protocols as needed based on the provided references.
2. Variablity in staining patterns may be observed across different cell types owing to distribution of specific lipids and cellular lipid composition.
3. The stringency of the fixation conditions can be adjusted by increasing the crosslinker concentration, i.e. 2% FA --> 4% FA --> 4% FA + 0.2% GA, as well as the temperature.
4. Most common crosslinking reagents are unlikely to react wtih lipids directly and will instead react mainly with proteins. As such, performing staining at 4 °C vs room temperature may affect residual lipid mobility after an initial fixation in some membranes.
5. Post-fixation of cells after antibody staining may also reduce residual lipid mobility.
6. In general, we do not recommend the use of acetone or methanol as cellular fixatives as they are organic solvents and can solubilize and extract lipids.
7. If the use of acetone or methanol is required to visualize a protein of interest during co-immunostaining for a specific lipid, then we encourage the user to compare the staining pattern for a given lipid with an alternative fixation procedure.
8. If using the 'general lipid staining ' protocol, the choice of permeabilization reagent should be made with the target

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cellular compartment in mind: digitonin and saponin are selective for cholesterol and therefore require it for effective permeabilization. Triton X-100 and Tween20 will permeabilize all membranes but may also completely solubilize some membranes such as the Golgi and ER.

9. At sufficiently high concentrations, Saponin, Triton X-100, and Tween20 may lyse cells or extract lipids. We do not recommend exceeding 0.5% Saponin, 0.2% Triton X-100, or 0.1% Tween20.

Protocol References

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