Immunofluorescence Protocol for use on cultured cell lines (IF-IC)

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Abstract

Goal:

Immunohistochemistry (or IHC) is a method for demonstrating the presence and location of molecules in cultured cells.

Solutions and Reagents:

NOTE: *Prepare solutions with deionized water or equivalent grade water. *Cells should be grown, treated, fixed and stained directly in multi-well plates, chamber slides or on coverslips.

1. 1X PBS
2. Paraformaldehyde 4%
3. Block solution: PBS + 1% BSA (Bovine Serum Albumin)
4. Block solution for intracellular antigen: PBS + 1% BSA (Bovine Serum Albumin) and Triton X100 0.001%.
5. Primary antibody, diluted in blocking solution.
6. Secondary antibody, conjugated to a fluorochrome, diluted in blocking solution.
7. 4',6-Diamidine-2'-phenylindoledihydrochloride (DAPI)
8. Mounting medium - Antifade

Coverslips preparation:

1. Immerse the coverslips overnight in absolute ethanol and then discard the ethanol.
2. Use a beaker with the coverslips in water and boil it 10 times (microwave) for a short period to eliminate ethanol residue.
3. Allow coverslips to dry completely and sterilize them in an autoclave.

Fixation of cultured cells:

1. Aspirate the cell medium, then cover cells with 250 µL of 1X phosphate-buffered saline (PBS). Gently shake.

2. Aspirate the 1X PBS

3. Add 4% paraformaldehyde in PBS pH 7.4. Allow cells to fix for 20 min at 4˚C (If necessary after

fixation the plates can be parafilm-sealed and kept at 4˚C in 1X PBS).

NOTE: Formaldehyde is toxic, use only in a fume hood.

4. Aspirate fixative, rinse two times in 1X PBS for 5 min each.

Blocking and immunostaining:

Note: All subsequent incubations should be carried out at room temperature unless otherwise stated, in a humid light-tight box or covered dish/plate to prevent drying and fluorochrome fading.

1. Block specimen in blocking buffer for 30 min. If the target protein is intracellular, it is very important to permeabilize the cells. If so, choose the blocking solution adequate for intracellular antigens.

2. While blocking, prepare primary antibody by diluting as indicated on datasheet in the same blocking solution buffer.

3. Aspirate blocking solution and then apply 250 µL of diluted primary antibody. For coverslips with a bigger diameter than 15mm, the volume should be adjusted to cover the whole area.

4. Incubate for 1 h at room temperature. Then aspirate the diluted primary antibody.

5. Rinse three times in blocking solution for 5 min each.

NOTE: If using a fluorochrome-conjugated primary antibody, cell nuclei should be labeled with DAPI and then skip to Step **10**.

6. Incubate specimen in 250 mL fluorochrome-conjugated secondary antibody diluted in block solution buffer with DAPI for 2 hr at room temperature in the dark.

7. Aspirate diluted secundary antibody.

8. Rinse three times in block solution for 5 min each.

9. Aspirate block solution, rinse three times in 1X PBS for 5 min each (500 mL).

10. Remove each coverslip from the cell culture plate with tweezers.

11. Invert the coverslip on to a glasslip with a drop of mounting media containing a fluorescence antifade agent.

12. Seal as required with nail polish to prevent drying and movement under the microscope.

13. For long-term storage, store slides flat at 4°C protected from light.

14. Examine the cells under a fluorescence microscope and image as required.

Reference

https://www.cellsignal.com/contents/resources-protocols/immunofluorescence-general-protocol/if

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