Immunocytochemistry Protocol

Preparation

In this protocol, we will prepare coverslips for immunofluorescent (IF) staining of adherent cultured cells. Coverslips are placed in 6-well tissue culture plates and later mounted on microscope slides for analysis. Most microscope objectives are designed to work best with #1.5 (0.17 mm thick) coverslips, which will give the best resolution and signal intensity. Acid cleaning will remove any dust which can cause artifacts, and coating them will improve the cell's adhesion to the glass.

1. Acid clean coverslips by incubating them in 1M HCl for 24 hours.

2. Rinse coverslips three times in laboratory grade water.

3. Rinse coverslips three times in 95% ethanol.

4. Prepare a 0.1 mg/ml solution of gelatin or poly-L-lysine and use a coverslip rack to submerge the coverslips for 5 minutes.

5. Air dry the covered slides in a culture hood, or place in a 37° C oven for faster drying.

6. Place the cleaned, coated, and dry coverslips into 6-well tissue culture plates and cover with lid.

7. Plates and coverslips can now be sterilized by placing under the hood's UV lamp for a minimum of 2 hours. Large volumes of plates can be prepared ahead of time for later culture.

Cell Growth and Fixation

1. Plate adherent cells at a density of 5 x10^5 per coverslipped well and culture overnight.

2. Aspirate culture medium and fix cells with 4% formaldehyde or 10% formalin for 10 minutes at room temperature (perform in fume hood).
3. Aspirate fixative and wash wells twice with PBS.

4. If probing for a target whose epitope is expressed intracellularly, cellular permeabilization is necessary.
    a. Nuclear/ mitochondrial localization: Incubate for 10 minutes with PBS containing 0.1-0.5% Triton X-100.

b. Cytoplasmic localization: Incubate for 10 minutes with PBS containing 0.1-0.5% Tween 20.

5. Aspirate permeabilization buffer and wash for 5 minutes three times with PBS-T (PBS with 0.1% Tween 20).

Blocking and Primary Antibody Incubation

1. Block unspecific binding sites with blocking buffer for 1 hour at room temperature. The best blocking buffer is PBS-T containing 10% serum from the host species of your secondary antibody, but 1% BSA in PBS-T may also be used.

2. Aspirate blocking buffer and add primary antibody diluted in blocking buffer. Use the recommended dilution of the antibody as specified on the datasheet. Incubate at 4° C overnight.

3. Aspirate primary antibody and wash for 5 minutes three times with PBS-T. Do not allow the cells to dry in this or any following step.

4. Prepare secondary antibody in blocking buffer and incubate for 1 hour at room temperature. Use the recommended dilution of the antibody as specified on the datasheet. Keep each plate covered with foil to prevent fading of the fluorophore from this step forward.

5. Aspirate secondary antibody and wash three times with PBS-T, for 5 minutes each.

6. Optional: Double/Nuclear labeling

    a. Double labeling: If using a second primary antibody and appropriately matched secondary, repeat steps 2-5.
    b. Nuclear labeling: After application of all primary antibodies, DNA binding dyes such as DAPI can be applied, which are used without the need for secondary antibodies. Use the recommended dilution and incubation time as specified on the datasheet. After incubation, wash once for 5 minutes with PBS.

6. Dispense one drop of anti-fade mounting medium onto a microscope slide. Remove the coverslip from the well with forceps and allow excess PBS to drip off. Mount coverslips (cells facing down) onto the slide.

7. Ring the edges of the coverslip with clear fingernail polish to prevent the cells from drying. Allow nail polish to air dry.

8. Slides may now be examined under a microscope with the appropriate fluorescent filter sets. Limiting the amount of time each slide is exposed to the microscope's light will aid in prolonging the signal and prevent photobleaching.

9. Slides can be stored between -20° C and 4° C in a dark slide box or slide book.

10. Slides can be stored between -20° C and 4° C in a dark slide box or slide book.