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IMMUNOFLUORESCENCE PROTOCOL

A. Cultured Cells

1. Coat coverslips with polyethylineimine or poly-L-lysine for 1 hr at room temperature.
2. Rinse coverslips well with sterile H2O (3 times 5 min each).
3. Allow coverslips to dry completely and sterilize them under UV light for at least 4 hrs.
4. Grow cells on glass coverslips or prepare cytospin or smear preparation.
5. Rinse briefly in phosphate-buffered saline (PBS).

B. Fixation

1. Fix the samples either in ice-cold methanol, acetone (1-10 min) or in 3-4% paraformaldehyde in PBS pH 7.4 for 15 min at room temperature.
2. Wash the samples twice with ice cold PBS.

C. Permeabilization

If the target protein is expressed intracellularly, it is very important to permeabilize the cells. Note: acetone fixed samples do not require permeabilization.

1. Incubate the samples for 10 min with PBS containing 0.25% Triton X-100 (or 100 µM digitonin or 0.5% saponin). Triton X-100 is the most popular detergent for improving the penetration of the antibody. However, it is not appropriate for the use of membrane-associated antigens since it destroys membranes.
2. Wash cells in PBS three times for 5 min.

D. Blocking and Incubation

1. Incubate cells with 1% BSA in PBST for 30 min to block unspecific binding of the antibodies (alternative blocking solutions are 1% gelatin or 10% serum from the species that the secondary antibody was raised in).
2. Incubate cells in the diluted antibody in 1% BSA in PBST in a humidified chamber for 1 hour at room temperature or overnight at 4°C.
3. Decant the solution and wash the cells three times in PBS, 5 min each wash.
4. Incubate cells with the fluorochrome-conjugate secondary antibody in 1% BSA for 1 hour at room

temperature in dark.

5. Decant the fluorochrome-conjugate secondary antibody solution and wash three times with PBS for 5 min each in dark.

E. Counter staining

1. Incubate cells on 0.1-1 μg/ml Hoechst or DAPI (DNA stain) for 1 min.
2. Rinse with PBS.

F. Mounting

1. Mount coverslip with a drop of mounting medium.
2. Seal coverslip with nail polish to prevent drying and movement under microscope.
3. Store in dark at -20 or 4°C.
4. Test under fluorescence microscope.