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IMMUNOFLUORESCENCE PROTOCOL FOR CELL CULTURE

This protocol is a recommendation only. Please optimize the procedure since experimental conditions can vary for different tissue samples.

SAMPLE PREPARATION

1. Coat coverslips with polyethylineimine or poly-L-lysine for 1 hour at room temperature.
2. Rinse coverslips with sterile H2O2 (3x5 minutes each).
3. Allow coverslips to dry completely and sterilize them under UV light for at least 4 hours.
4. Grow cells on glass coverslips or prepare cytospin or smear preparation.
5. Rinse briefly in tris-buffered saline (TBS).

FIXATION

6. Fix the cells in either 3-4% paraformaldehyde in TBS (pH7.4) or ice-cold acetone or methanol for 15 minutes at room temperature.
7. Wash the samples twice with ice cold PBS.

PERMEABILIZATION

If the target protein is intracellular it is important to permeabilize the cells. Cells that have been fixed with acetone do not require permeabilization.

8. Incubate the samples for 10 minutes in TBS containing 0.25% Triton X-100 (or 100uM digitonin or 0.5% saponin).
9. Wash cells in TBS 3 times for 5 minutes each.

BLOCKING AND INCUBATION

10. Incubate cells with blocking buffer for 30 minutes to block unspecific binding of the antibody.
11. Incubate the cells with the primary antibody overnight at 4°C (in the dark if using a fluorescent primary).
12. Remove the solution and wash cells 3 times with TBS for 5 minutes each.
    Note: Skip to step 15 if using a primary conjugated antibody.
13. Incubate the cells with the secondary antibody for 1 hour at room temperature in the dark.
14. Remove the solution and wash 3 times with TBS for 5 minutes each in the dark.

COUNTERSTAINING AND MOUNTING

15. Incubate the cells with DAPI for 1 minute.
16. Rinse with TBS.
17. Mount coverslip with a drop of mounting medium.
18. Store in the dark at -20°C or 4°C.