

## IMMUNOCYTOCHEMISTRY (ICC)

### General Procedure:

- i Coat coverslips with polyethyleneimine or poly-L-lysine for 1 hr at room temperature.
- ii Rinse coverslips well with sterile H<sub>2</sub>O (3 times 5 min each).
- iii Allow coverslips to dry completely and sterilize them under UV light for at least 4 hrs.
- iv Grow cells on glass coverslips or prepare cytopspin or smear preparation.
- v Rinse briefly in phosphate-buffered saline (PBS).

### 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.

### Permeabilization:

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

3. 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.
4. Wash cells in PBS three times for 5 min.

### Blocking and Incubation:

5. 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).
6. Incubate cells in the diluted antibody in 1% BSA in PBST in a humidified chamber for 1 hr at room temperature or overnight at 4 °C.
7. Decant the solution and wash the cells three times in PBS, 5 min each wash.
8. Incubate cells with the secondary antibody in 1% BSA for 1 hr at room temperature in dark.
9. Decant the secondary antibody solution and wash three times with PBS for 5 min each in dark.

### Counter staining:

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

### Mounting:

12. Mount coverslip with a drop of mounting medium.

13. Seal coverslip with nail polish to prevent drying and movement under microscope.
14. Store in dark at -20 or 4 °C.