Immunocytochemistry (ICC) Protocol

1. Wash the wells with PBS.
2. Add cold Paraformaldehide %4, 20 min at 4˚C and then 5 min at RT.
3. Wash the wells with cold PBS twice.
4. Triton X100 %0.4, 5-15 min (depending on the antigen), RT; and then wash with PBS (For intracellular antigens only)
5. Add gout serum 5%, 45 min, RT; then remove it, do not wash.
6. First antibody (diluted in BSA/PBS 0.2%), 4˚C, overnight.
7. Wash the wells with PBS-Tween %0.1, 3×5 min.
8. Add BSA/PBS 1%, 30 min, RT; then remove it, do not wash.
9. Second antibody (diluted in BSA/PBS 0.2%), 3 h, RT.
10. Wash the wells with PBS-Tween %0.1, 3×5 min.
11. Add PBS to the wells, keep in dark.

Notes:

***** During washing handle each well individually, since leaving a washed dish without medium for even a few seconds can allow drying in the center of the well.

*****Handle the solutions gently to prevent detachment of the cells.

*****For the analysis of cells with Fc receptors, the use of isotype-matched negative control is mandatory.

*****For Nuclear staining add DAPI (1 ug/ml) for 10 min. (DAPI is a strong carcinogenic!)

*****Keep Fluorescent antibody in dark.

*It is preferred to filter all of the solutions before use to avoid a dirty background.

Materials:

PBS (pH=7.4)

PBS-Tween %0.1

Triton X100 %0.4 and %0.04

Paraformaldehide %4 (pH=7.4)

BSA/PBS 1%

Gout serum 5%