

IMMUNOFLUORESCENCE – SPLENOCYTES

1. Put poly-L-lysine coated coverslips in 6 well plates
2. Incubate at least 10^6 cells, in its media, on the poly-L-lysine coated coverslip for at least 2 hours in the TC incubator (maximum 4 hours).
3. Wash the cells X 2 with PBS (Add 5mL of PBS and aspirate away from the slip)
4. Add 3 mL of Fixative (3% NBF (free 10% from histology), 2% sucrose in PBS) for 10 minutes at room temperature
5. Wash the cells X 2 with PBS
6. The cell can be stored, at this point, in PBS for months. For storage tightly wrap the plates with parafilm to avoid evaporation.
7. If not stored, incubate with 3mL of 0.1% Triton-X in PBS for 10 minutes.
8. Wash the cells X 2 with PBS
9. Add 2%FBS in PBS for at least 1 hour at room temp
10. Remove the 2% FBS in PBS and incubate the cells with the primary antibody at 4 deg overnight. Parafilm the plate to prevent evaporation and drying. Primary antibodies are to be diluted with 2%FBS in PBS. Minimally, the coverslip need to be covered with the antibody.
11. The next day, aspirate the antibody
12. Wash the cells X 2 with 2% FBS in PBS
13. Remove the 2%FBS in PBS and incubate the cells with the appropriate secondary antibody in a dark place, at room temp for 30 minutes. Secondary antibodies are to be diluted with 2%FBS in PBS. Minimally, the coverslip need to be covered with the antibody.
14. Wash the cells X 2 with 2% FBS in PBS
15. Wash the cells with PBS

Poly-L Lysine solution

Dilute 1:10 and submerged desired number of coverslips in solution. Remove solution from coverslips by aspiration, and allow to fully dry before use.

Fixative

Dissolve 1g sucrose in 15 mLs 10% NBF (free from histology)
Bring final volume up to 50 mLs with 1x PBS

Make fresh for each use, and discard according to appropriate chemical disposal procedures for fixatives

Blocking solution

Add fetal calf serum to 1X PBS for a final concentration of 2% (v/v)