**Immunofluorescence of macrophages and microglia**

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**Abstract**

This protocol describes the fixation and staining of cultured cells in paraformaldehyde solution.

**Keywords**

Immunofluorescence, antibody

**Solutions to prepare**

**Fixative solution:** 4% paraformaldehyde in 0.2 M phosphate buffer pH 7.4.

**Permeabilization and locking solution:** 0.1% (v/v) saponin + 5% (w/v) BSA in PBS

**Primary antibody solution**: 0.1% (v/v) saponin + 5% (w/v) BSA in PBS solution

**Secondary antibody solution:** 0.1% (v/v) saponin + 5% (w/v) BSA in PBS solution

**Protocol**

1. Plate 50,000 iPSC derived macrophages or microglia or Neurons or BMDM’s cells on 22 x 22 mm glass coverslips in 24-well dishes in respective culture media
2. Incubate overnight at 37°C in 5% CO2
3. Pre-warm a solution of 4% paraformaldehyde to RT.
4. Aspirate media from cells and immediately proceed to step 5.
5. Add pre-warmed 4% paraformaldehyde to cells.
6. incubate for 15 minutes at room temperature.
7. Remove 4% paraformaldehyde solution and dispose of it in an appropriate chemical waste container.
8. Rinse each well with 3X 1 mL PBS. Remove rinse and dispose of it in an appropriate chemical waste container.
9. Add 0.1% (v/v) saponin + 5% (w/v) BSA in PBS and incubate for 60 minutes at room temperature to permeabilize cells.
10. Aspirate 0.1% (v/v) saponin + 5% (w/v) BSA in PBS solution.
11. Prepare respective antibody dilutions in 0.1% (v/v) saponin + 5% (w/v) BSA in PBS solution. mouse LAMP1 (DSHB #ID4B) 1:200 dilution, TFE3 (Sigma #HPA023881), 1:200 dilution.
12. Incubate at 4°C overnight.
13. Aspirate antibody solution and wash cells with PBS for 5 minutes.
14. Repeat wash with 3X PBS for 5 minutes.
15. Add filtered PBS containing 5% BSA and secondary antibodies (1:600, Alexa fluorophores 488 and 555, Invitrogen).
16. Incubate at room temperature for 1 hour.
17. Aspirate antibody solution and wash cells with PBS for 5 minutes 3X.
18. Rinse coverslips in milliQ water.
19. Mount coverslips onto slides using ProLong Gold Antifade Mountant with DAPI (ThermoFisher P36935).
20. Allow slides to cure overnight in darkness.
21. Image.