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Immunofluorescence of macrophages and microglia

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We use this protocol and it's working

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Abstract

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

Attachments

DOCX

mqjvbj3up.docx

16KB

Materials

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

LAMP-1 (human) **Developmental Studies Hybridoma Bank Catalog #**ID4B

Anti-TFE3 antibody produced in rabbit **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**HPA023881

ProLong™ Gold Antifade Mountant with DAPI **Thermo Fisher Catalog #**P36935

Immunofluorescence of macrophages and microglia

3h 55m

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% CO_2.

3 Pre-warm a solution of 4% paraformaldehyde to Room temperature.

4 Aspirate media from cells and immediately proceed to step 5.

5 Add pre-warmed 4% paraformaldehyde to cells.

6 Incubate for 00:15:00 at Room temperature.

15m

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 01:00:00 at Room temperature to permeabilize cells.

1h

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.

1h

13 Aspirate antibody solution and wash cells with PBS for 00:05:00 . 5m

14 Repeat wash.

14.1 Wash with PBS for 00:05:00 . (1/3) 5m

14.2 Wash with PBS for 00:05:00 .(2/3) 5m

14.3 Wash with PBS for 00:05:00 .(3/3) 5m

15 Add filtered PBS containing 5% BSA and secondary antibodies (1:600, Alexa fluorophores 488 and 555, Invitrogen).

16 Incubate at Room temperature for 01:00:00 . 1h

17 Aspirate antibody solution and wash cells.

17.1 Wash cells with PBS for 00:05:00 . (1/3) 5m

17.2 Wash cells with PBS for 00:05:00 . (2/3) 5m

17.3 Wash cells with PBS for 00:05:00 . (3/3) 5m

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.

5m

21 Image.