Apr 17, 2025

Immunocytochemistry

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DOI
https://dx.doi.org/10.17504/protocols.io.dm6gpdoz1gzp/v1

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Protocol Citation: Lucas Blasco-Agell, Veronica Testa, Valentina Baruffi, Antonella Consiglio, Miquel Vila 2025. Immunocytochemistry. **protocols.io** https://dx.doi.org/10.17504/protocols.io.dm6gpdoz1gzp/v1

Protocol status: Working
We use this protocol and it's working

Created: April 17, 2025

Last Modified: April 17, 2025

Protocol Integer ID: 130937

Keywords: detection of key microglia marker, ipscderived human microglia cell, key microglia marker, human microglia cell, immunocytochemistry immunocytochemistry

Funders Acknowledgements:
ASAP
Grant ID: ASAP-020505

Abstract

Immunocytochemistry (ICC) detection of key microglia markers in iPSCderived human microglia cells (hMG)

1 Cells plated in 24-well plates are fixed before any ICC staining using 4% of Paraformaldehyde (PFA; EMS) at RT for 15 minutes. Excess of PFA is removed from the culture wells with three washes of 15 minutes with DPBS.

2 For whole vmO IN TOTO ICC, samples are fixed in PFA at 4ºC for 2 hours and rinsed 3 times with TBS for 20 minutes.

3 For ICC of cryosectioned vmO, samples are fixed in 4% PFA at 4ºC Overnight (ON). Fixed vmO are transferred to 30% Sucrose (Sigma-Aldrich) solution at 4ºC ON.

4 vmO are placed on plastic molds with O.C.T.™ compound (Tissue-Tek®) at -80ºC until processing. Cryopreserved vmO were sectioned (20 m thick) on a cryostat (Leica) and mounted in Menzel-Glaser Superfrost® PLUS coverslips (Thermo Fisher Scientific), dryed at RT ON and stored at -20ºC for further ICC staining.

5 To stain hMG, we used a low Triton X-100 (Triton; Sigma-Aldrich) protocol. Coverslips are placed on Menzel-Glaser Superfrost® Microscope slides (Thermo Fisher Scientific) inside a humid chamber.

6 Samples are then rinsed in 1X Tris-Buffered Saline (TBS) before being blocked at RT for 2 hours with Blocking Solution (BS), which consisted of TBS with 0.01% of Triton and 3% of Donkey Serum (DS). After that, samples are incubated with primary antibodies at 4ºC for 48 hours.

7 Samples are rinsed and blocked again with BS at RT for 1 hour and incubated with secondary antibodies for 2 hours at RT protected from light. All secondary antibodies employed are from Alexa Fluor Series (Jackson ImmunoResearch Europe) at a concentration of 1:250.

8 After that, samples are rinsed TBS and nuclei are counterstained with 0.5 µg/mL of 4,6-Diamidino-2-phenylindole (DAPI; Abcam) at RT for 10 minutes.

9 Samples are mounted within a glass coverslip (Corning®) with Polyvinyl Alcohol-1,4-Diazabicyclo-Octane (PVA-DABCO; Sigma-Aldrich).

10 Mounted slides are dried at RT for 30 minutes protected from light and stored at 4ºC before analysis. To stain vmDAn, 2D co cultures or vmO cryosections, we used a high Triton protocol except that rinses are done with TBS with 0.1% of Triton and BS consists of TBS with 0.3% of Triton and 3% of DS.

11 For the whole organoid structures, *IN TOTO* ICC staining is performed. vmO are rinsed with TBS and incubated with an antigen retrieval buffer composed by Sodium Citrate Tribasic (Sigma-Aldrich) with a pH of 9 at 60ºC for 1 hour.

12 vmO are rinsed in TBS at RT in agitation (Labnet International) and permeabilized with TBS plus 0.5% Triton and 6% DS at 4oC ON in agitation. Samples are then incubated with primary antibodies, diluted in BS (TBS with 0.1% Triton and 6% DS) at RT at 4ºC over-the-weekend in agitation.

13 After 3 days, samples are rinsed with BS at RT and incubated with secondary antibodies (diluted in BS) at 4ºC ON in agitation.

14 The day after, vmO are rinsed TBS and nuclei are counterstained with 2.5 µg/mL DAPI (diluted in TBS). Samples are mounted with PVA-DABCO in a glass coverslip, dried and stored at 4ºC for further imaging. Images are acquired using Zeiss AXIOIMAGER Z1 with an ApoTome (Zeiss Microscopy) microscope using a Cascade CCD camera (Photometrics). For confocal images, Zeiss AXIOIMAGER Z1 with an SPE confocal system or a Zeiss LSM 880 are employed (Zeiss Microscopy). Image acquisition is realized with ZEN pro software (Zeiss Microscopy) and subsequently analyses are performed using ImageJ (NIH), Photoshop® (Adobe) and IMARIS (Bitplane copyright).