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Olfactory mucosa immunostaining

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

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Disclaimer

Abstract

This protocol describes the immunostaining of olfactory mucosa cells after cocnentrating and depositing a monolayer of the cell suspension onto a slide. For the preparation of the olfactory mucosa suspension refer to protocol "Olfactory mucosa sampling and processing" : **dx.doi.org/10.17504/protocols.io.14egn2b86g5d/v1**

Materials

- benchtop centrifuge with cooling function
- Cytospin centrifuge and signle use plastic funnels
- regular lab equipment (pipetts, tips, tubes)
- Glass slide racks and containers (optional)
- NaCl 0.9% (w/v) aqeous solution
- Whatman paper (thick)
- PAP Pen
- Glass slides
- PBS
- BSA
-Triton-X100

Troubleshooting

Preparation of OM suspension

1 Follow the protocol for olfactory mucosa collection and extraction of the cells from the nasal swab. Centrifuge the cell suspension at 2000 x g for 20 mins at 4 °C and resuspend the cells in 1mL 0.9% NaCl.

00:00:00

2 Count the number of cells in the suspension and adjust the cell concentration to 80-120.000 cells per mL.

Cytospin

3 Label glass microscope slides, assmble the glass slides, single-use cytospin funnels and funnel clips in the cytospin centrifuge. Ensure that the cytospin centrifuge is balanced and pipette 100 µL of the cell suspension into each cytofunnel. This will ensure a monolayer of 8-12.000 cells.

4 Set the cytospin centrifuge at 500 x g for 15 mins.

Cell fixation and immunostaining

5 After centrifugation let the cell monolayer dry on the slide for a couple of minutes and draw around it a hydrophobic barrier with a PAP pen.

6 Fix cells by applying a drop of 4% PFA and incubate for 15 mins at RT. (For many slides this step can be performed with a staining rack in a fixation bath.)

7 After fixation, perform two wahses with PBS by carefully removing the solution from the slide with a thick Whatman paper and applying the PBS drop on top of the cells. (For many slides this step can be performed with a staining rack in a PBS bath.)

8 Make sure the PBS from the washes is completely removed and perform blocking-permeabilization step by applying 3% BSA, 0.1% Triton-X100 in PBS for 1 hr at RT.

9 Dilute the primary antibodies of choice in the appropriate concentrations in blocking-permeabilization buffer and apply on top of the cell monolayer after completely removing the solution from the previous step. Incubate overnight at 4°C.

10 Perform two wahses with PBS by carefully removing the solution from the slide with a thick Whatman paper and applying the PBS drop on top of the cells. (For many slides this step can be performed with a staining rack in a PBS bath.) At this stage ensure the hydrophobic circle around the cells is intact and re-apply if necessary.

11 Dilute the secondary antibodies and nuclei staining of choice in the appropriate concentrations in blocking-permeabilization buffer and apply on top of the cell monolayer after completely removing the solution from the previous step and incubate for 1 hr at RT in the dark.

12 Perform two wahses with PBS by carefully removing the solution from the slide with a thick Whatman paper and applying the PBS drop on top of the cells. (For many slides this step can be performed with a staining rack in a PBS bath.)

13 After the washes carefully remove the hydrophobic barrier and apply a mounting medium of choice and a coverslip on top of the cell monolayer. Let it dry and store in the dark until imaging.