

Jul 20, 2023

Olfactory mucosa immunostaining

DOI

dx.doi.org/10.17504/protocols.io.x54v9pyeqg3e/v1

Mary Xylaki¹

¹University Medical Center Goettingen



Mary Xylaki

UMG

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account





DOI: https://dx.doi.org/10.17504/protocols.io.x54v9pyeqg3e/v1

Protocol Citation: Mary Xylaki 2023. Olfactory mucosa immunostaining . protocols.io

https://dx.doi.org/10.17504/protocols.io.x54v9pyeqg3e/v1

License: This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: July 20, 2023



Last Modified: May 31, 2024

Protocol Integer ID: 85270

Keywords: Cytospin, Olfactory mucosa, Immunostaining, ASAPCRN, immunostaining of olfactory mucosa cell, preparation of the olfactory mucosa suspension refer, olfactory mucosa suspension refer, olfactory mucosa cell, olfactory mucosa sampling, olfactory mucosa, immunostaining, monolayer of the cell suspension, cell suspension, protocol, cell

Disclaimer

DISCLAIMER - FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

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

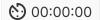
- benchtop centrifuge with cooling function
- Cytospin centrifuge and signle use plastic funnels
- regular lab equipment (pipetts, tips, tubes)
- Glass slide racks and containers (optional)
- NaCl 0.9% (w/v) ageous solution
- Whatman paper (thick)
- PAP Pen
- Glass slides
- PBS
- BSA
- -Triton-X100

Troubleshooting



Preparation of OM suspension

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.



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

Cytospin

- 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 μ L 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

- 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.
- 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.)
- 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 blockingpermeabilization step by applying 3% BSA, 0.1% Triton-X100 in PBS for 1 hr at RT.
- Dilute the primary antibodies of choice in the appropriate concentrations in blockingpermeabilization 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.