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Immunohistochemistry (Brain organoids)

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Anita Adami¹

¹Lund University



Laura Castilla-Vallmanya

Lund University

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We use this protocol and it's working

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Abstract

This protocol describes how to prepare brain organoids slides and how to perform immunostainings to study the expression and localization of target proteins in this 3D model.



Sample Preparation

4h

- 1 Organoids collected for staining were transferred into a 24-well plate and fixed with 4% paraformaldehyde (PFA) for 02:00:00 .
- 2 Fixed organoids were then rinsed twice in DPBS and immersed in a 1:1 OCT (catalog no. 45830, HistoLab) and 30% sucrose emulsion in which they were incubated Overnight at 4 °C on gentle shake.
- 3 On the following day, the organoids were transferred to a cryomold in OCT and frozen on dry ice, to be stored at -80 °C until cryosectioning.
- 4 Organoids were cut on a cryostat at -20 °C into sections of 20 um thickness and placed onto Superfrost Plus microscope slides.

Immunohistochemistry

3h 30m

- 5 A hydrophobic PAP Pen was used to draw the borders of the slides.
- 6 Slides were then washed twice in 1X TKPBS (PBS + 0.3% Triton X-100) and fixed again with 4% PFA for 00:10:00 .
- 7 After two washes with KPBS for 00:10:00 , slides were immersed in Blocking solution (TKPBS + 5% Normal Donkey Serum) for at least 01:00:00 .
- 8 300 ul of Primary antibody solution (blocking solution+ primary antibodies at the chosen dilution) were added to each slide and these were incubated Overnight at 4 °C in a humidified chamber.
- 9 On the following day, slides were washed three times with KPBS for 00:10:00



- 10 300 ul of Secondary antibody solution (blocking solution + secondary antibodies at the chosen dilution, 1:1000 DAPI) were added to each slide and these were incubated for at least 01:00:00 at Room temperature protected from light.
- 11 Slides were then washed three times with KPBS for 00:10:00
- 12 Slides were mounted with FluorSave mounting medium, allowed to dry at Room temperature and then stored at 4 °C until imaged.
- 13 Slides were imaged on a confocal Microscope Leica TSC SP8 and images were cropped and adjusted on ImageJ Fiji.