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Immunohistochemistry (neural organoids)

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We use this protocol and it's working

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Abstract

This protocols describes how to perform immunohistochemistry on neural organoids

Troubleshooting

Fixing and mounting

4h

1 The organoids were fixed in 4% paraformaldehyde for 02:00:00 at Room temperature . 2h

2 They were subsequently washed three times with KPBS and left in a 1:1 OCT:30% sucrose solution and OCT (HistoLab) mixture Overnight . 2h

3 The organoids were then transferred to a cryomold containing OCT and frozen on dry ice and stored at -80°C.

Sectioning

4 Prior to the staining protocol, the organoids were cryosectioned at a thickness of 20 µM.

Staining

2h 10m

5 For immunohistochemistry, sections were washed in PBS1X for 00:10:00 and then blocked and permeabilized in **Blocking solution** (0.3% Triton X-100 and 4% normal donkey serum in PBS1X) for at least 01:00:00 . 1h 10m

6 The sections were then incubated with primary antibodies in **blocking solution** at 4 °C Overnight . 1h

The primary antibodies used for neural organoids characterisation were rabbit anti-PAX6 (1:300; BioLegend, Cat# 3700, RRID:AB_2242334)) and rat anti-ZO1 NB110-68140, RRID:AB_1111431(1:300; Novus)).

7 After incubation with the primary antibodies, the sections were incubated for 1 h with the appropriate secondary antibodies (Alexa Fluor 488, 594, 647 used at 1:400; Molecular Probes).

Mounting

8 Finally, the sections were mounted on gelatin-coated slides and coverslipped with PVA-DABCO containing DAPI (1:1000).