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Immunofluorescence staining and image analysis

In 1 collection

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We use this protocol and it's working

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Abstract

Immunofluorescence staining and image analysis

2D cultures IF

1 Seed cells on coverslips and fix with 4% paraformaldehyde (PFA) for 10 minutes

2 Rinse cells with phosphate-buffered saline (PBS)

3 Block cells with 10% normal goat or donkey serum (NGS/NDS) in PBS containing 0.1% Triton X-100 (PBST) at room temperature for 1 hour

4 Incubate cells overnight at 4°C with primary antibodies diluted in PBST containing 5% NGS/NDS

5 The next day, after washing with PBS 3 times for 5', incubate cells for 2 hours at room temperature with Alexa 488/568/647-conjugated secondary antibodies at a 1:1000 dilution, diluted in PBST containing 5% NGS/NDS

6 Remove secondary antibodies and stain nuclei with 1 µM DAPI for 5 minutes

7 Wash coverslips 3 times for 10'

8 Mount coverslips on glass slides with Dako mounting medium

Brain organoids and Assemblods IF

9 For brain organoids and assembloids, fix samples with 4% (w/v) PFA for 1 hour

10 Equilibrate organoids in 30% sucrose in PBS overnight at 4°C

11 Embed organoids in OCT compound the following day

12 Cryosection organoids to 20 µm thickness and mount on SuperFrost Plus slides

13 Block sections with 10% NGS in PBS containing 0.5% Triton X-100

14 Incubate sections with primary antibodies diluted in PBST containing 5% NGS/NDS overnight

15 After washing sections with PBS 3 times for 5' Incubate with secondary antibodies diluted in PBST containing 5% NGS/NDS for 3 hours

16 (If BODIPY staining is needed, incubate cells or tissues with 1 µM BODIPY 493/503 dye in PBS for 30 minutes at room temperature)

17 (For protein aggregate detection, stain sections with 10 µM thioflavin-T for 30 minutes at room temperature)

18 Stain sections with 1 µM DAPI for 5 minutes

19 Wash sections three times with PBS for 10'

20 Mount sections with Dako mounting medium

Mouse tissue IF

21 For mouse brain tissue, perform antigen retrieval on free-floating brain slices for 20 minutes in citrate buffer at 80°C

22 Block tissues with mouse-on-mouse (MOM) blocking reagent following manufacturer's instructions

23 Incubate with primary antibodies overnight using MOM protein concentrate

24 After washing in PBS 3 times 5', incubate with secondary antibodies for 3 hours

25 Stain sections with 1 µM DAPI for 5 minutes

26 Wash sections three times with PBS for 10'

27 Mount sections with Dako mounting medium

Image analysis

28 Acquire images using a Leica TCS SP8 confocal microscope with a 60×/1.4 numerical aperture objective or an EVOS M7000 Imaging System with a 20×/0.4 numerical aperture

29 For quantification, analyze p21-, p16-, and γH2AX-positive nuclei in CellProfiler with DAPI as a counterstain

30 Calculate fluorescence intensity per area using Fiji