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

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# Immunostaining of Organoid Sections

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ABSTRACT

Immunostaining of Organoid Sections derived from human iPS cells.

MATERIALS

- Blocking Buffer: lxPBS + 5% FBS + 1% BSA+ 0.3% Triton X + 0.1% Azide
- lx PBS
- 24-well plate
- Paint brush
- Incubation Buffer: lxPBS + 1% Triton X
- Primary and secondary antibodies
- Fluoromount with DAPI

1 Place organoid sections into 1ml of PBS in a 24-well plate (1 section per well)

2 Aspirate off the PBS gently

3 Add ~600ul of blocking buffer for 20mins at RT on the rocker 20m

4 Wash 3x with 500ul of PBS and aspirate off PBS

5 Add primary AB at working concentration and incubate in Blocking Buffer overnight at 4°C (GFAP-1:500)

6 Wash 3x with 500ul of PBST, and aspirate it off

7 Add secondary AB at working concentration and incubate in Blocking Buffer at RT on a rocker for 1 hour, making sure to cover the plate with aluminum foil to prevent bleaching of the secondary AB (1:1000 for both secondaries)

8 Wash 3x in PBST mount onto a slide in water

9 DAPI staining is 2ug/ml in PBST for 15 min at RT 15m

10 Wash 3x with PBST

11 Once the section is on the slide, aspirate off some of the water and add a drop of Fluoromount with DAPI bef ore placing round coverslip on top, being careful not to cause any bubbles underneath

12 leave overnight at RT to let dry and covered to prevent bleaching

13 Store covered at 4C