Organoid Immunohistochemistry Protocol

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Freezing and embedding

1. Cut tip off of P200ul tip and move organoids to a 1.5ml Eppendorf tube
2. Remove media and wash twice with 1ml PBS.
3. Fix in cold 4% PFA for 30 min on shaker at 4C.
4. Wash three times with 1ml PBS
5. Add 1ml 30% sucrose to tube and incubate at 4C for 1hr or until organoids settle to the bottom.
6. During incubation, prepare molds using 2-ply aluminum foil.
7. Prepare ethanol:dry ice slurry.
8. Remove sucrose and wash once with 1ml PBS.
9. Cut tip off of P200 tip. Set the P200 pipetteman to 100ul.
10. Coat the P200 tip with FBS. Pick up all organoids at once and let them settle to the bottom of the tip. Gently push down on pipetteman throttle until the organoids collect in a droplet at the bottom of the cut off tip.
11. Gently touch the droplet to the bottom of the aluminum foil mold. The organoids should stick and you should minimize the amount of PBS that enters mold. If there is too much PBS, carefully remove excess using P200.
12. Cut off the tip of a P1000. Add 900ul of OCT/30% sucrose mix (2 parts OCT to 1 part 30% sucrose) to the aluminum foil mold containing the organoids.
13. Using P200 tip, swirl the organoids in the OCT/30% sucrose mix. This dilutes PBS and separates the organoids from each other.
14. Place mold in ethanol:dry ice slurry making sure that the ethanol does not get into the mold. The OCT should freeze and turn bright white within two minutes.
15. Place mold into a 24-well plate and store at -80C until sectioning. Once sections are cut, you can PFA fix for <5 min.

Immunostaining

1. Outline the sections using a PAP pen. Allow to dry for 1-2 minutes.
2. Fix sections with 4% PFA for 5 minutes at RT in glass chamber.
3. Wash 3 times with 1X PBS for 5 minutes at RT in glass chamber.
4. Block/permeabilize for 1hr at RT in a humified chamber:
    a. 50ml 10% Donkey Serum in PBS
    b. 0.38g glycine
    c. 150ul Triton X-100
    d. 1 drop of Image-IT
5. Add primary antibody in 1% Donkey Serum in PBS. Incubate for 1hr at RT or shaking O/N at 4C.
6. Wash 3x5 minutes in a glass chamber in 1X PBS + 0.05% TritonX-100.
    a. 100 ml PBS
    b. 500uL of 10% TritonX-100
7. Add secondary antibody in 1% Donkey Serum + 1:1000 DAPI for 2-3hr shaking at RT.
8. Wash 3x5 minutes in a glass chamber in 1X PBS + 0.05% TritonX-100
9. Wash 2x5 minutes in 1X PBS
10. Set out to dry briefly. Add 50-100uL of Fluoromount to each organoid and add coverslip. Seal with clear nailpolish.

Cultured Cells Immunocytochemistry Protocol

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1. Remove cells from incubator and gently wash once with 1X PBS (150ul for 96-well plates, 500ul for 24-well plates). Manually aspirate rather than using vacuum if cells are not firmly attached to the dish.

2. Add 4% PFA to each well (50ul for 96-well plates, 400ul for 24-well plates) and incubate at room temperature for 15 minutes.

3. Add 1X PBS to each well (150ul for 96-well plates, 1ml for 24-well plates) to dilute PFA. Then wash three times with 1X PBS to completely remove PFA.

4. Add 0.1% Triton (50ul for 96-well plates, 400ul for 24-well plates) to permeabilize cells and incubate for 10 minutes at room temperature.

5. Remove 0.1% Triton and add blocking solution (10% Normal Donkey Serum diluted in 1X PBS; 50ul for 96-well plates, 400ul for 24-well plates) for 1 hour at room temperature.

6. Remove blocking solution and add primary antibody diluted in blocking solution (30ul for 96-well plates, 300ul for 24-well plates). Incubate overnight at 4C.

7. Wash three times with 1X PBS well (150ul for 96-well plates, 1ml for 24-well plates).

8. Add secondary antibody diluted in blocking solution and incubate in the dark for 1-2 hours at room temperature.

9. Wash once with 1X PBS (150ul for 96-well plates, 1ml for 24-well plates).

10. Add DAPI solution (1:10,000 in 1X PBS; 50ul for 96-well plates, 400ul for 24-well plates) and incubate in the dark for 5 minutes.

11. Wash two times with 1X PBS well (150ul for 96-well plates, 1ml for 24-well plates).

12. The samples are now ready for imaging. If the cells were on a coverslip, they can now be mounted onto a glass slide.