Systems *Bioengineering* Laboratory

Organoid Immunofluorescent Staining Protocol

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Materials

- 4% Paraformaldehyde Fixative
  - PFA Stock, 16% Paraformaldehyde (EMS, Cat: 15710)
  - Sucrose (Sigma, Cat: S7903)
  - 10X PBS (Gibco, Cat: 70011-044)
  - Mix 26 mL of ddH_2O, 4 mL of 10X PBS, 10mL PFA stock and vortex mix. Store at 4ºC and use for up to two weeks.
- Blocking Buffer (Antibody Diluent)
  - Bovine serum albumin (Sigma, Cat: A8806)
  - Donkey Serum (Sigma, Cat: S30-M)
  - 0.3% Triton-X 100 (Sigma, Cat: X100) diluted in PBS
  - Prepare and aliquot sterile filtered 10% BSA stock in ddH_2O. Dilute 1mL stock with 500 µL 10X PBS, 250 µL donkey serum and 3.25 mL of ddH_2O. Add 0.3% Triton-X 100. Store at 4ºC and use for up to two weeks.
- Hoechst 33342 (Life Technologies, Cat: H3570)
- 96 Well Optical Bottom Plate (Life Technologies, Cat: 165305)
- 35mm Glass bottom dish (Cellvis, Cat: D35-14-1.5-N)

Methods

1. After 18+ days organoid culture, move the organoids to an optical bottom plate, aspirate the media.
2. Fix the organoids with *4% paraformaldehyde fixative* (200µL) at room temperature (RT) for 1 h.
3. Aspirate the PFA and wash with PBS (200µL) at RT three times.
4. Replace PBS with *blocking buffer* (400µL) and incubate in RT for 3 h.
5. Prepare primary antibody by diluting antibody of choice with *blocking buffer* (200µL) at the recommended ratio.
6. Replace *blocking buffer* with primary antibody and incubate for overnight at 4°C
7. Aspirate primary antibody and add PBS (200µL). Allow to incubate for 1 h. Repeat.
8. Aspirate the 2nd PBS wash and add new PBS (200µL). Allow to incubate for 3 h or overnight at 4°C.
9. Prepare secondary antibody by diluting antibody of choice with *blocking buffer* (200µL) at 1:200. Cell stains such as LTL or DBA maybe added along with secondary antibody at 1:200 dilution.
10. Replace PBS with secondary antibody and incubate at RT for 2h.

11. Replace thrice with PBS (200µL), incubating ~1 min between each wash.
12. Replace PBS with Hoechst 33342 (200µL) at 1:5,000 in PBS. Incubate the organoids at RT for 30 mins.
13. Replace thrice with PBS (200µL), incubating ~1 min between each wash.
14. Leave in PBS and image in optical bottom plate or Celvis glass bottom dish.