

## Fixing organoids for Histology

### Recovery of organoids from culture

**All centrifugation steps done for 5 minutes @ 1600rpm, 4°C unless otherwise stated.**

1. Recover organoids from Matrigel domes.

○ In plates:

- Remove media.
- Add **500µL room temperature PBS** per well, swirl gently to wash.
- Remove PBS and add **500µL organoid recovery solution** per well
- Disrupt each gel dome w/ pipette tip.
  - Disrupt dome w/ swirling/mixing motion.
- Transfer contents into 15mL tube, pooling wells from each sample into corresponding tubes.
- Add **500µL cold media** to each well, scrape with pipette tip, and move to the same tube to ensure complete removal of well contents.

○ In 15mL tubes:

- Top **total volume** of tube up to **10mL with cold media or PBS**.
- Centrifuge samples.
- Remove supernatant with glass/serological pipette, leaving all gel in pellet.
- Add **1mL of organoid recovery solution** to each tube (unless its contents represent >4 wells, then add 2mL) and pipette up and down to resuspend gel into solution
- Incubate organoids in **organoid recovery solution for 75 minutes** in fridge.

2. Move each sample and its organoid recovery solution into 15mL tubes if not in them already. Bring **total volume** of the tube **up to 10mL with cold PBS**.
3. Centrifuge samples.
4. Remove supernatant with glass/serological pipette and aspirator.
5. Resuspend organoids in **2mL of cold PBS** with p1000 pipette.
6. Centrifuge samples.
7. Carefully remove as much supernatant as possible with p1000, followed by p200 pipette.
8. Add **1mL of 10% neutral buffered formalin** per tube and incubate for 10 minutes at room temperature.
9. Top volume of each tube up to **10mL with room temperature PBS** and centrifuge.
10. **Quickly** decant supernatant into formalin-specific waste stream by inverting the tube.  
  
Resuspend the organoids in **1mL of PBS** with p1000, then top total volume **up to 10mL with PBS**. Centrifuge.
11. Repeat step 9.
12. Resuspend organoids in **1mL of 70% ethanol with p1000**, then top total volume **up to 3-5mL with 70% ethanol**.
13. Allow organoids to settle to bottom of tubes.