



Spence Laboratory - University of Michigan
Alyssa Miller - 6/12/2018

Whole Mount Staining

Methods:

Note: perform all steps on a gentle rocker or nutator.

1. Fix tissue for 30-60 minutes in 4% PFA
2. Wash in 1X PBS (2 short [on/off], 2 long [30 minutes])
3. Block with 5% NDS 0.1% PBST for 2.5 hours
4. Incubate in 1° AB overnight (24 hours preferably)
5. Wash in PBST for **at least** 5 hours (may need to increase wash time to reduce background)
6. Incubate in 2° AB + DAPI overnight
7. Wash in PBST (2 short, 2 long)
8. Dehydrate in methanol (PBST with concentration of methanol) ~15 minutes
 - 25% to 50% to 75% to 100% MeOH
 - NO ETHANOL
9. Transfer organoid onto slide with cavity and allow methanol to evaporate. Add Murry's clear to coverslip to image.
 - Alyssa's advice: Add all over and fill cavity. Then, tilt slide towards you before coverslipping. If seal is not ideal, add nail polish.
 - Store in methanol