## General protocol: Fluorescent Immunohistochemistry (IHC):

DAY ONE:

1. Label all slides w/ **pencil**.
    - For organoids: do not use slide warmer as it disrupts organoid integrity.
    - For tissues: slide warmer can be used but may cause sections to fall from slides more easily.
2. Dewax slides by incubate slides in series of 2 buckets of **xylenes** for 10 minutes each, 2 buckets of **100% ethanol** for 5 minutes each, and 2 buckets of **95% ethanol** for 5 minutes each.
3. Incubate slides in bucket of **distilled water** for 10 minutes
4. Move slides to a bucket of appropriate **antigen retrieval solution***, place bucket in steamer, and steam for 20 minutes to 1hr.
5. After antigen retrieval steam, allow slides to cool in freezer, then transfer to a bucket of room temperature distilled water.
    - Monitor slides/bucket during cooldown for freezing/ice formation.
    - While slides cool, dilute antibodies in diluent of **casein buffer.**
        - Casein blocks nonspecific antibody binding.
        - Aim for **200µL of diluted antibody per slide/sample.**
    - Place moistened paper towels inside of humidity chamber and flatten them within grooves to accommodate slides.
6. Fill 3 buckets w/ **250mL 1x PBS** (dilute from 10x stock). Add 1% Tween to the first bucket. Transfer slides in rack & wash for 5 minutes in each bucket (PBS-Tween → PBS → PBS)

- *If using TLAQ:* detergents will wash away TLAQ and therefore are incompatible. All steps involving detergents should be completed prior to TLAQ application.
- *If using TLAQ:* during washes, prepare **200µL** of TLAQ working solution per slide/sample by **diluting stock solution 1:20 in 70% EtOH**.

7. Working one slide at a time:
    1. Blot water off of slides at a site far from the sample.
    2. Draw a wide circle (at least 1cm gap between circle + organoids) around sample with hydrophobic pen.
        a. Do not press too hard with pen.
        b. Ensure each circle is complete.
    3. Allow pen to dry very briefly then transfer slide into humidity chamber.
    4. *If using TLAQ:*
        a. Starting with 1 slide: set timer to keep track of intervals between each slide; prepare **250mL of PBS** for wash.
        b. Add **200µL of TLAQ** to slide.
            i. If necessary, gently disperse antibody solution with pipette tip just contacting the solution itself, not touching the sample/slide!
            ii. TLAQ is <u>light sensitive.</u> Slides should be kept from light sources as much as possible once TLAQ has been applied.

c. Repeat for all slides while being mindful of the interval between each slide.
    i. Specific interval does not matter as long as they are consistent between slides.
    ii. Keep the slides in a line in the order they TLAQ for ease of tracking.
d. Following incubation of 3-5 minutes w/ TLAQ, transfer each slide into PBS per your time intervals and order.
    i. This ensures that each slide incubates with secondary antibody for a full 1 hour.
    ii. Prepare 2 additional buckets of **250mL PBS**.
e. Wash for 5 minutes in each of the 3 PBS buckets.
f. Repeat step 7.1 for each slide prior to step 7.5

5. Apply primary antibodies:
    a. Add **200µL of diluted primary antibodies** per slide/sample.

8. Place lid on humidity chamber and carefully move the chamber to the +4ºC fridge for overnight incubation of primary antibodies.