**Whole Mount Immunofluorescence (WMIF) for organoid cultures**

Modified from Muthuswamy Lab protocol by Tina

1. Plate organoids in MG dome accordingly. Plan to fix for IF 2-3 days post-plating.
2. Aspirate media from chambers.
3. Quick wash with 250ul of 1xPBS.
4. Fix cells with 2% PFA or \*5% formalin at RT for 20 minutes.
5. Wash 3x with 1xPBS/Glycine solution for 10 minutes each, gently rocking (2 rpm).

\*Note: If fixing with 5% formalin, a permeabilization step is needed here.

 5b) Permeabilize with 0.5% TritonX100/PBS for 10 minutes.

1. Wash 3x with 1xIF Wash solution for 10 minutes each, gently rocking.
2. Incubate in block (1xIF + 10% goat/horse serum) for 1-1.5 hours gently rocking.

Note: Can do overnight at RT.

1. Aspirate block and incubate in primary antibody diluted in block for 1-4 hours, gently rocking.

Note: Can do overnight at RT.

1. Aspirate primary antibody solution.
2. Wash 3x with 1xIF Wash solution for 10 minutes each, gently rocking at RT.
3. Incubate with secondary antibody diluted in block (1xIF wash + 10% goat/horse serum) for 1 hour, gently rocking at RT.

Note: Keep in dark from this point forward.

1. Wash 2x with 1xIF Wash solution for 20 minutes each, gently rocking at RT.
2. Incubate with DAPI (1:10,000) in 1xPBS for 10 minutes, gently rocking at RT.
3. Wash at least 1x with 1xPBS for 10 minutes, gently rocking at RT.
4. Mount with mounting medium in chambers (see “Mounting Medium for Organoids”protocol; can be stored in -20˚C for several months), or coverslip with Cytoseal and allow time for it to dry before imaging.

**Solutions:**

10x PBS/Glycine (500ml)

38.0g NaCl

9.38g Na2HPO4 (sodium phosphate dibasic anhydrous)

2.07g NaH2PO4 (sodium phosphate monobasic)

37.5g glycine

pH 7.4 and filter sterilize

10x IF-wash (500ml)

38.0g NaCl

9.38g Na2HPO4 (sodium phosphate dibasic anhydrous)

2.07g NaH2PO4 (sodium phosphate monobasic)

2.5g NaN3

5.0g BSA (Fraction V)

10ml Triton X-100

2.5ml Tween-20

pH 7.4 and filter sterilize