

## ***Indirect Immunofluorescent Staining of MCF-10A Acini Cultured In Matrigel (Simplified)***

*Day 1: Follow immunofluorescence protocol through primary antibody – incubate primary antibody overnight.*

- 1) Aspirate media and fix cells with 400µl of 2% Formalin or 3-4% PFA for 20 minutes at RT.
- 2) Permeabilize cells with pre-chilled 0.5% Triton X-100 in PBS for 10 minutes at 4°C.
- 3) Rinse with PBS-Glycine 3 times, 10 minutes each
- 4) Incubate with 200 µl of primary block (1X IF buffer + 10% goat serum) for 45-60 minutes.
- 5) **OPTIONAL:** Aspirate the block and incubate with secondary block (1X IF buffer + 10% goat serum + 1:100 dilution of F(ab')) for 30-40min
- 6) Incubate with primary antibody in block O/N at 4°C (most antibodies used at 1:100-200 in 200µl /well)

*Day 2:*

- 1) Allow chamber slides to come to room temperature (this will make the Matrigel bed more rigid and less likely to be aspirated during washing steps).
- 2) Rinse with 1X IF buffer 3 times, 20 minutes each
- 3) Incubate with secondary antibody in 1X IF buffer + 10% goat serum for 45-50min at RT (1:200 in 100-200 µl/well). Keep slides in the dark from this step forward.
- 4) Rinse with 1X IF buffer 1 time, 20 minutes
- 5) Rinse with PBS 2-3 times, 10 minutes each
- 6) Incubate with 200 µl of 5µM TOPRO-3 in PBS for 15 min at RT. (Can also incubate with DAPI (1µg/ml final) in PBS for 15 min at RT alone or simultaneously with the TOPRO).
- 7) Rinse 1 time with PBS, 5 minutes
- 8) Aspirate PBS, score the adhesive between the plastic chambers and slide with a razor blade.
- 9) Lift plastic chambers from glass slide: place the chamber slide in a black holder and slide a white lifter through the holder until its edge touches the edge of the wells. Gently pull up the chambers (holder and lifter comes with BD Falcon chamber slides).
- 10) Aspirate any residual liquid from edges of the slide.
- 11) Mount with Prolong anti-fade (Molecular Probes) – use one small drop/per chamber, regardless of whether the chamber is empty.
- 12) **OPTIONAL:** Warming the slide at 37°C for 10-25 minutes can help the Prolong spread evenly across the slide.
- 13) Dry slides in the dark O/N at RT. Once Prolong is dried completely, slides can be sealed with nail polish and stored at 4°C for up to two weeks or at –20°C for several months.