

Immunofluorescence of Mammary Epithelial Acini

Day 1:

Cells don't need to be rinsed prior to fixation. Aspirate media and fix cells with 400ul 2% PFA (in PBS PH7.4; less than 1 week at 4C or fresh thaw) for 20 minutes at RT or 2% formalin for 20 minutes. You can also fix using 1:1 mix of methanol:acetone at -20C for 10-20 minutes. (Once fixed, slides can be stored at 4C for up to one week. Rinse wells once in PBS and store in 500ul/well PBS. Wrap wells in Saran wrap to avoid drying out.)

Permeabilize cells with 0.5% Triton in PBS for no longer than 10 minutes at 4C

Rinse 3X with 1x PBS-Glycine for 10 minutes each

Incubate with 200ul of primary block (IF Wash + 10% goat serum) for 1-1.5 hrs

Aspirate the serum block and incubate with secondary block (IF + 10% goat serum + 1:100 dilution of F(ab)₂ (1.3 mg/ml stock – Jackson Lab) for 30-40min (optional step)

Incubate with primary antibody in secondary block O/N at 4°C (most antibodies used at 1:100-200 in 100-200ul /well)

Day 2:

Allow chamber slides to heat up to room temperature (this will harden the Matrigel bed and make it less easy to aspirate during washing steps).

Rinse 3X with IF Wash (gentle rocking) for 20 minutes each

Incubate with secondary antibody (Alexa Fluor -- Molecular Probes) in IF + goat serum for 50-60min at RT (1:100 in 100-200ul/well). Keep slides in the dark from this step until finish.

Rinse once at RT with IF Wash for 20 minutes

Rinse 2X with PBS (pH 7.2-7.4) for 10 minutes each

Incubate with TOPRO-3 in PBS for 20-30 min at RT -- 1:100 dilution in 500ul OR
Incubate with DAPI (1µg/ml final) in PBS for 20 min at RT --1:10,000 dilution of 10mg/ml stock

DAPI and TOPRO-3 can also be incubated simultaneously for 20-30 minutes at RT.

Rinse once with PBS.

Lift plastic chambers from glass slide by cutting in between with a razor blade, placing the chamber slide in a black holder and sliding a white lifter through the holder until its

edge contacts the edge of the wells. Gently pull up the chambers (holder and lifter comes with BD Falcon *CultureSlide*).

Mount with Prolong anti-fade (Molecular Probes) and allow to dry O/N at RT. Once Prolong is dried completely, slides can be wrapped in foil and stored at 4C for two weeks or at -20°C for several months.

10X PBS/Glycine:

38.0 g NaCl
9.38 g Na_2HPO_4
2.07 g NaH_2PO_4
37.5 g Glycine

10X IF Wash:

38.0 g NaCl
9.38 g Na_2HPO_4
2.07 g NaH_2PO_4
2.5 g NaN_3
5.0 g BSA
10.0 ml Triton-X 100
2.05 ml Tween-20

Bring both solutions up to a total volume of 500 ml, pH 7.4

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