BMES Cell Team 2019-2020: Lab 3 Immunostaining Protocol

*Volumes are for a 24 well plate

Day 1

1. Fix cells
    a. Add 500uL 4% paraformaldehyde to each well
    b. Incubate for 15 mins
    c. Perform in fume hood because PFA is TOXIC!
2. Wash with PBS-Tween
    a. 500uL per well
    b. Wash three times for five minutes each
3. Add 500uL of Triton to each well
    a. Incubate for 5 minutes
4. Wash with PBS-Tween
    a. 500uL per well
    b. Wash three times for five minutes each
5. Block with 500uL 5% BSA-PBS-Tween for an hour an hour at RT
6. Add primary antibodies
    a. Do not add primaries to one well of each sample so they can act as the control
    b. Rabbit anti-SOX2 diluted added at 1:200
    c. Add 500uL to each well x 10 wells = 5mL
        i. Will not make extra because antibodies are expensive
        ii. 5mL PBS-Tween + 25uL Rabbit anti-SOX2
    d. Incubate at 4C overnight

Day 2

1. Wash with PBS-Tween
    a. 500uL per well
    b. Wash three times for five minutes each
2. Add secondary antibodies
    a. When making, cover in foil to avoid photobleaching
    b. Three secondaries
        i. Hoescht -- stains nuclei (stains all cells)
        ii. Goat anti-Rabbit, -- binds to Rabbit anti-SOX2
        iii. Phalloidin -- binds to actin, already fluorescent
        iv. Add Hoescht and Goat anti-Rabbit to all wells, add Phalloidin only to wells with primaries
    c. Wells without primary (control wells)
        i. Add 500uL to each well x 2 wells = 1mL
        ii. 1mL PBS-Tween + 1uL Hoescht + 1uL Goat anti-Rabbit
    d. Wells with primary
        i. Add 500uL to each well x 10 wells = 5mL

        ii. 5mL PBS-Tween + 5uL Hoescht + 5uL Goat anti-Rabbit + 10uL Phalloidin
    e. Incubate at RT covered in foil for one hour
3. Use EVOS microscope to image
    a. Turn off lights to avoid photobleaching
    b. Look at cells under phase (transmitted) at 4x
    c. Look at fluorescent channels
        i. Hoescht -- DAPI
        ii. SOX2 -- GFP
        iii. Actin (Phalloidin) -- RFP
            1. View at 40x to see different actin cytoskeleton morphology between cell lines