Indirect Immunofluorescence for Pluripotency Markers

1. Grow ES/iPS cells on 8-well chamber slides.
2. Fix cells in 4% paraformaldehyde/PBS (-Mg, -Ca) for 10 min at room temperature.
3. Wash cells 2x with PBS. Can store cells at this point in PBS at 4C up to 1 week before processing.
4. Permeabilize cells with 0.2% Triton X-100/PBS for 10 min at RT.
5. Wash 3x (5 min each) with PBS.
6. Block with blocking solution (5% donkey serum/PBS) for 60 min at RT.
7. Dilute primary antibodies to working concentrations in blocking solution.
8. Incubate with 100 ul of primary antibody overnight at 4C (place chamber slides in moist container to avoid evaporation).
9. Wash 3x (5-10 min each) with 0.2%Tween-20/PBS.
10. Dilute secondary antibody in PBS to working concentrations.
11. Incubate with 100 ul secondary antibody for 60 min at RT.
12. Wash 3x (5-10 min each) with PBS.
13. Aspirate the last wash and apply a drop of antifade w/DAPI and mount slide with coverslip.
14. Seal slide with clear nail polish.
15. Store slides in the dark to avoid bleaching.

Solutions/reagents:
<u>4% PFA (Toxic: Dissolve in the chemical hood!!!)</u>
For 1 L:

1. Add 400 ml of H2O to a 2L glass beaker. Heat H2O to 64C on a hot plate.
2. To 980 ml of PBS in the 1L plastic PBS bottle, add 100 ul 10N NaOH, 40 g PFA and a stir bar.
3. Put the PBS bottle into the heated beaker containing water and stir until PFA is dissolved and solution clears (when the solution reaches 64C).
4. Remove the 4% PFA solution from the hot plate and cool to RT.
5. Filter sterilize and aliquot to store at -20C.

Donkey serum: Millipore S30-100ML
Antifade with DAPI: Invitrogen S36938