Immunostaining

(cells on coverslips)

Steps

1) Seed and treat cells on coverslips in a 6-well plate (or smaller format).
2) Wash coverslips with 1x PBS (all washes and volumes 1ml); fix with 4% paraformaldehyde/1xPBS for 0.5h at room temperature.
3) Permeabilize with 0.5% Triton X-100/1x PBS for 5 minutes.
4) Wash 2x with 1xPBS, 5' per wash.
5) Block with 3% BSA/PBS for 1h at room temperature (or overnight at 4ºC).
At this stage prior to primary antibody incubation, transfer cells onto parafilm that can be protected from light (for example, a slidebox).

6) Dilute primary antibody 1:500 or 1:1000 in 1%BSA/1xPBS, incubate 1h at room temperature. Small coverslips, can use a 50ul volume, and invert coverslpis onto parafilm (cell-side down onto the parafilm).
7) Place coverslips cell-side up on parafilm, wash with 1xPBS 3 times, 5' per wash.
8) Add 2º antibody, 1h at room temperature in 1%BSA/PBS (Alexa conjugates at 1:250), as well as any other staining reagents such as phalloidin (i.e. Alexa 568-phalloidin, 1:50 in 1%BSA/PBS - 1:250 okay). Invert coverslips onto parafilm (cell-side down), incubate at room temperature 20-30 minutes. At this stage on, protect the cells from light.
9) Wash with 3x with PBS, 5' – cell side up on parafilm.
10) Add DAPI 1:2000 in 1%BSA/PBS at room temperature, 10' (stained separately due to short staining duration). Can skip this step if using mounting media containing DAPI.
11) Wash with 1xPBS, 2x briefly (1st wash, 5-10', 2nd wash rinse)
12) Seal with Prolong anti-fade kit.

Methanol permeabilization for cofilin rods (Huang et al., Dev Cell 2009):
Fix in 4%PFA/0.1% Glutaraldehyde for 45 mins., the fix in cold methanol (-20°C), 3 mins. at -20ºC.
Block in 3% BSA/1x PBS, and follow the remainder of the staining protocol.

Note: cannot use methanol fixation for phalloidin staining!