

IMMUNOFLUORESCENCE

1. Plate cells on glass coverslips that have been acid-washed treated.
2. Allow cells to grow for 2 days.
3. Wash cells 2x with cold 1xPBS (aspirate off supernatant).
4. Fix cells for 10 min. at room temperature in 2% paraformaldehyde (add about 10 ml to a 100x20mm plate). PA is prepared by adding the PA powder to 1xPBS that has been heated in the microwave to about 65°C. Be careful when you add it; it is caustic and will burn your eyes! Add the PA to the PBS in the hood; let it cool somewhat and then adjust the pH to 7.2. Allow PA to cool until it reaches RT.
5. Wash cells 2-3x with cold 1xPBS.
6. Permeabilize cells by incubating in ice cold, absolute methanol for 3-5 min. at -20°C.

Another method used to permeabilize the cells is using 0.5% Triton X-100/1xPBS for 5 min.

7. Wash cells 2-3x with cold 1xPBS.
8. Incubate cover slips in filtered 1% BSA/1xPBS for 30-60 min. to prevent non-specific staining.
9. Set up a humid chamber for the antibody incubations: Using a 150x20mm petri dish, place a kim-wipe on the bottom and cover it with dH₂O using a squirt bottle (but don't flood the dish). Cover the kim wipe with a sheet of parafilm (a 4 square piece works perfectly).
10. Wash cells 2-3x with cold 1xPBS.
11. Using a jewelers forceps, pick up an individual coverslip (*important: pay attention to which side of the coverslip the cells are on!*) and gently wipe off any excess liquid from the back with a kim-wipe; also, hold the coverslip horizontal to pull off any final drops of liquid with the kim-wipe (but be careful not to disturb the cells). Set the coverslip down "cell-side up" on the parafilm in the humid chamber.
12. Add 50 µl of the primary antibody (diluted in filtered 1xPBS with 1% BSA) to coverslip and incubate for 30-40 min. at 37°C in humidified atmosphere.
13. Wash 2x with 1xPBS (rinsing each coverslip individually by holding it with the forceps in a small beaker of 1xPBS). Again, pay attention to the orientation of the cells. Wipe off excess liquid from the back and side of the coverslip before placing it back into the humid chamber.
14. Add 50 µl preabsorbed, fluorescent-tagged secondary antibody (we use the Texas red-conjugated goat anti-rabbit IgG from Molecular Probes at a dilution of 1:500) to the coverslips and incubate for 30 min. at 37°C in humidified atmosphere.
15. Wash coverslips 2-3x in PBS; rinse briefly with distilled water (important! to rinse off any residual PBS to prevent salt crystals from forming).
16. Place an 8 µl drop of Mowoil (Calbiochem #475904) onto a microscope slide. Mount the coverslip to the slide "cell-side down" on top of the Mowoil.
17. The coverslips can be viewed under the microscope.