Immunostaining Protocol FH April 2010

This is a protocol developed for staining alpha tubulin (Abcam, ab52866) and gamma tubulin (Sigma T6557) in IMCD cells, based on Attias 2010, doi:10.1159/000262317. It can be used as a reference, but depending on your antibodies you might consider other fixation protocols such as ice cold methanol or acetone. Dilutions of antibodies have to be determined by titration.

When using multiple primaries at the same time, primary’s host must be of different species.

biocompare.com is a useful resource of commercially available antibodies

Example: primary: rabbit anti alpha tubulin IgG, secondary donkey anti rabbit IgG Alexa 555

Materials:

Circular cover glass 12 mm 1.5   
 Triton X-100  
 Paraformaldehyde (PFA)  
 PBS  
 Bovine Serum Albumin  
 Prolong Anti Fade mounting medium  
 Primary and secondary antibodies

Preparation:

1. Plate cells on 12mm circular 1.5 glass coverslips the day before in 24 well plate (50’000 cells/well)
2. Prepare 0.5% Triton X-100 in PBS *(Triton X is very viscous, so prepare a few days before and make serial dilutions)*
3. Prepare 4% Paraformaldehyde in PBS
   1. Perform all handlings inside fume hood and wear gloves (PFA is toxic!)
   2. Heat PFA in PBS to 60°C on heating plate and use magnet to stirr
   3. PFA dissolves and solution becomes clear
4. Prepare 3% Bovine Serum Albumin (BSA) in PBS

Staining:

1. Wash cells twice with prewarmed PBS
2. Fix cells with 500 uL 4% PFA in PBS for 15 min at RT in fume hood
3. Wash 3x with PBS *(discard all washes into toxic waste container)*
4. Permeabilize cells with 500 uL 0.5% Triton-X in PBS for 5 min
5. Wash 3x with PBS
6. Block with 3% BSA in PBS for 30 min at RT
7. Prepare primary antibodies (dilute in 0.5 % BSA in PBS)
8. Discard blocking solution, add primary antibodies
   1. Either overnight at 4°C (decreases background)
   2. 1-2 h at room temperature
9. Wash 3x 20 min with PBS
10. Add secondary antibodies (dilute in 0.5% BSA in PBS)
11. Wash 3x 20 min with PBS
12. Mount with ProlongAntiFade mounting medium, let it cure for 24 hours at room temperature protected from light
13. Seal the coverslips with nailpolish