Immunofluorescence of HEK Cells Protocol

1. Transfect HEK293 cells with plasmids and incubate at 37oC with 5% CO2 for to 24-48hrs allow for protein expression
2. Remove media and wash cells in 1xPBS
3. Fix cells in cold methanol and incubate at RT for 20 min
4. Wash samples 3x in 1xPBS
5. Prepare humid chamber by placing wetted paper in large petri dish and apply samples
6. Permeabilize cells in 1xPBS with 0.1% Triton-x100 and incubate for 30mins at RT
7. Wash samples 3x in 1xPBS
8. Block for 1hr at RT in Blocking solution (1mg/ml BSA, 10mM NaN_3 in 1xPBS)
9. Dilute primary antibody in Blocking solution and incubate at RT for 1hr (or at 4^0C O/N)
10. Wash 3x in 1xPBS
11. Dilute secondary antibody (1:500 dilution of Alexa Fluor 488, 568 or 633 antibodies) in Blocking solution and incubate at RT for 1hr (samples must be kept in dark so cover with foil)
12. Wash 3x in PBS
13. Add a drop of Vectashield (with DAPI) on microscope slide and place coverslip-containing the cells on top
14. View samples on fluorescence microscope and acquire images on confocal microscope