Fluorescent staining of cultured mammalian cells

Actin (cytoskeleton) staining with Alexa Fluor 488-phalloidin (Molecular Probes) – fixed cells

What do we need for this procedure?

- AF488-phalloidin reconstituted in methanol
- 1XPBS (phosphore buffered saline)
- precooled 4% formaldehyde
- 0.1% Triton X-100
- 1% BSA (bovine serum albumine)
- DAPI – nuclear dye

Note:

1. Cells (HTC-75 cell line, human fibrosarcoma) are grown on cover slips in wells of 24-well plate;
2. each step (washing, incubation)should be performed in R_T.

Procedure:

1. Remove the medium from the wells by using a vacuum pump.
2. Fix the cells with (fresh!) 4% FA (0.5 ml), for 5 min; wash 5x with 1XPBS (0.5 ml each time).
3. Transfer the cover slips on glass slides covered with parafilm.
4. Permeabilize the cell membrane for 5 min with 0.1% Trition X-100 (40 ul); wash 3x with 1XPBS (80 ul each time).
5. Block 30 min in 1% BSA (40 ul); remove the solution thoroughly, do not wash!
6. Incubate with AF488-phalloidin working solution (5 ul in 200 ul of PBS); wash 5x with 1XPBS.
7. Counterstain 5 min with DAPI working solution; wash 1x with 1XPBS.
8. Coverslip with a drop of PBS.
9. Check under the microscope☺.

Plasma membrane staining with FM 4-64 lipophilic styryl dye (Molecular Probes) – live cells

What do we need for this procedure?

- FM 4-64 dye, 8 uM
- nuclear dye for live cells – NucBlue® Live ReadyProbes® Reagent (Hoechst 33342)

Note:

1. Cells (the same as above) are grown in glass chamber slides;
2. each step should be performed in R_T.

Procedure:

1. Prepare a working staining solution of FM 4-64 dye (1 mM from 10 mM, 10 ul, in ice-cold 1XPBS).
2. Remove the cells in chamber slides from the incubator; add 2.4 ul of 1mM FM 4-64 dye per well (final concentration of the dye is 8 uM).
3. Incubate 1 min, in 4^{o}C.
4. Add a drop of NucBlue® Live ReadyProbes® Reagent.
5. Visualize immediately!