Cell Lines and Culture Conditions

Unless otherwise noted, all human cell lines are cultured in complete medium and grown in a 37˚C humidified incubator with 5% CO2.

What you need in advance

1. Human cell lines of interest.
2. Disposable 25 cm^2 and 75 cm^2 tissue culture flasks and 6-well plates.
3. Complete medium: Dulbecco's Modified Eagle Medium supplemented with 10% heat-inactivated fetal bovine serum (FBS).
4. Trypsin: Trypsin-EDTA solution 0.25%. Can be prepared by adding 2.5 g porcine trypsin and 0.2 g EDTA.4Na to one litre of Hanks` Balanced Salt Solution
5. Sterile phosphate-buffered solution (PBS), pH 7.4.
6. Disposable sterile pipettes (1, 5, and 10 mL).
7. Disposable conical tubes (15 and 50 mL).

Transient Plasmid DNA and Small Interfering RNAs (siRNAs) Transfections

If you are planning on transfecting your cell prior to imaging, this is what you require:

1. Complete medium.
2. Reduced serum medium: DMEM supplemented with 5% FBS.
3. Opti-MEM
4. Lipofectamine 2000.
5. Lipofectamine RNAi Max.
6. Plasmid DNA of interest or siRNA oligos.
7. Solution A:
    i. For DNA: Dilute 5 µL of Lipofectamine 2000 in 250 µL of Opti-MEM.
    ii. For siRNAs: Dilute 5 µL of Lipofectamine RNAi Max in 250 µL of Opti-MEM.
8. Solution B:

i. For DNA: Dilute 1 ng/mL of the plasmid DNA of interest in 250 µL of Opti-MEM.
ii. For siRNAs: Dilute 2µL of the siRNA of interest (Stock Concentration = 20 mM; Final Concentration = 20 nM) in 250 µL of Opti-MEM.

9. Selection antibiotics, depending on the plasmid to be used (e.g., for HeLa cells, G418 at 200-400 µg/mL, puromycin at 1-2 µg/mL).

Requirement for Live-Cell Imaging

1. Spinning disk microscope.
2. Magnetic imaging chamber (Live Cell Instrument).
3. 6-well plates.
4. FluoroDishes 35 mm well, glass-bottom 0.17 mm thickness (World Precision Instruments).
5. Glass coverslips: 22 x 22mm No. 1.5.
6. Complete medium
7. Imaging medium: Leibovitz's L-15 medium supplemented with 10% FBS.
8. Antibiotics (e.g., penicillin-streptomycin solution, antibiotic-antimycotic solution).

Transient Plasmid DNA and Small Interfering RNAs (siRNAs) Transfections

Day 0

1. Seed the human the cell line of interest in a 6-well plate well with 2 mL of complete medium.
2. Grow cells in a 37˚C humidified incubator with 5% CO_2, until they are 50–75% confluent.

Day 1

3. Rinse the cells with sterile phosphate-buffered solution (PBS) and replace the cell culture medium with 1.5 mL of reduced serum medium.
4. Incubate cells in a 37˚C humidified incubator with 5% CO_2.

5. For cell transfection, prepare solutions A and B (see **Notes [5] and 6**).
6. Incubate solutions A and B separately for 5 min at room temperature (RT).
7. Mix solutions A and B and incubate for 30 min at RT.
8. Add the oligo-lipid complexes to the cells, dropwise.
9. Incubate the cells for 5-6 h in a 37˚C humidified incubator with 5% CO2.
10. Replace the culture medium with 2 mL of complete medium.
11. Grow cells in a 37˚C humidified incubator with 5% CO_2 for 24, 48, or 72 h (see **Note 7**).
12. Analyse cells (see **Note 8**).

Seeding Cells on FBN-patterned Glass Coverslips for imaging

1. Incubate the patterned face of the coverslip with 50-200 µL of FBN and fluorescence-conjugated fibrinogen for 1 h, at RT.
2. Rinse the coverslips with PBS.
3. Harvest human cell line of interest by washing with PBS and incubating with the Trypsin solution for 2min. Trypsin volume will vary according to the surface area (e.g., for a 25mm^2 flask, use 2 mL of Trypsin and for a 6-well plate well, use 1 mL of Trypsin).
4. Seed 50,000 to 200,000 cells in the FBN-patterned coverslips with 2 mL of complete medium supplemented with antibiotics.
5. Incubate the cells in a 37˚C humidified incubator with 5% CO_2 for 1–2 h.
6. Gently wash the unattached cells with complete medium.
7. Grow cells in a 37˚C humidified incubator with 5% CO_2 for 12-24 h.
8. Image cells on the microscope (see **below)**.

Live-Cell Imaging

Day 0

1. 12-24 h prior to each experiment, seed 50,000 to 200,000 cells (depending on cell type) in FBN-patterned coverslips with 2 mL of complete medium supplemented with antibiotics.

Day 1

2. Approximately 30 min to 2 h prior to each experiment, assemble the magnetic imaging chamber and replace the cell culture medium with 1-2 mL of imaging medium supplemented with antibiotics. Add SiR-dyes or drugs of interest (acute pharmacological inhibition) to the cell culture medium at this time point.
3. Turn ON all the imaging equipment and set the appropriate temperature and CO_2 levels (37˚C and 5% CO2) for the microscope chamber.
4. Set up your experimental conditions, namely the appropriate illumination wavelengths, laser power intensities, exposure times and time-lapse conditions.
5. Start imaging.