Culture of Primary Human Endothelial Cells

Fibronectin coating of culture surface

1. Dilute stock solution of fibronectin (1:100) with PBS, and add the solution (150 μl/cm^2) to culture dish/flask.
   For T-75 flask, add 100 μl fibronectin stock solution to 10 ml PBS.
2. Leave the dish/flask in a CO_2 incubator at 37 ^oC overnight.
3. Aspirate fibronectin solution, and wash with PBS once.

Thawing frozen cells

1. Place the vial in a 37 ^oC waterbath, hold and rotate the vial gently until the contents are completely thawed.
2. Remove the vial from the waterbath immediately, wipe it dry, rinse the vial with 70% ethanol and transfer it to a sterile field.
3. Remove the cap, being careful not to touch the interior threads with fingers. Using a pipette gently re-suspend the contents of the vial.
4. Add the contents to 10 ml culture medium in a 15-ml conical centrifuge tube.
5. Centrifuge 5 min at 1,500 x g, and carefully aspirate the supernant without disturbing the cell Add 1 ml culture medium to cell pellets, and gently re-suspend the cells by pipetting up and down.
6. Add culture medium (0.2 ml/cm^2) to dish/flask, and add suspended cells (5,000 – 10,000 cells/cm^2) to the culture medium.
   For T-75 flask, add ~500,000 cells in 15 ml medium.
7. Gently mix the medium and place the dish/flask in a CO_2 incubator at 37 ^oC.

Cell culture and maintenance

1. Change the medium to fresh supplemented medium every 3 days, until the culture is approximately 70% confluent.
2. Subculture the cells when cells reach 70% - 90% confluent.

Cell subculture

1. Rinse cells with PBS once.
2. Add trypsin/EDTA solution (50 μl/cm2); gently rock the flask to make sure cells are covered by trypsin/EDTA solution; Immediately aspirate the solution.
   For T-75 flask, add 4 ml trypsin/EDTA solution.
3. Incubate the cells in an incubator at 37 ^oC for 1 to 2 min or until cells are completely rounded up (monitored with inverted microscope). At the end of trypsinization, one hand hold one side of flask, and the other hand gently tap the other side of the flask to detach cells; check the flask under inverted microscope to make sure all cells are detached.
4. Add 10 ml culture medium and gently resupend the cells by pipetting up and down.
5. Count cells and plate cells in a new, fibronectin-coated flask (10,000 cells/cm^2).
   A typical subculture rate is 3-5 (the cells in one flask are divided and cultured in 3-5 flasks).

Appendix

1. Fibronectin stock solution: fibronectin (Sigma #F4759, 1 mg/ml) in PBS, aliquot and store at -80 ^oC.
2. Cell culture medium (Lonza #CC-3162, EGM-2 with SingleQuote supplement; or ScienCell #1001, ECM medium with 5% FBS, or #1001-prf phonel red-free).
3. Trypsin/EDTA solution: 0.05% trypsin and 0.5 mM EDTA in PBS.