

ISOLATION OF HUMAN DERMAL ENDOTHELIAL CELLS

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Neonatal foreskins are transported in DMEM or HBSS with pen/strep, gentamicin and anti-PPLO.

1. Wash twice with DMEM to remove blood.
2. Transfer to a petri dish containing DMEM.
3. Cut into small fragments. Very important!
4. Wash twice with DMEM.
5. Incubate in collagenase (~5mg/ml) in DMEM at 37 °C for 30 min, mixing with a stir bar.
6. Filter the solution through 70 um filter.
7. Centrifuge to collect the cells.
8. Incubate with trypsin (0.05%) in versene at 37 °C for 15-30 min.
9. Add media with serum to stop the reaction.
10. Centrifuge to collect the cells.
11. Resuspend in complete media and plate in a vitrogen-coated petri dish (~2 foreskins/1 p60).
12. After 2-3 hours, wash with media to remove contaminant cells (endothelial cells should be attached).

PURIFICATION

Between 3-4 days after the isolation, taking care that the fibroblast doesn't start overgrowing the endothelial cells.

1. Incubate the cells with TNF-alfa (100 ng/ml) or LPS (100 ng/ml) for 4-5 h.
2. Add 10-20 ul of anti-ELAM-1 coated dynabeads to the cells and incubate 10-15 min, checking under microscope. Mix the beads well before adding.
3. Wash 3 times with DMEM.
4. Trypsinize the cells.
5. Insert the tube with the cells into magnet for 2 min (or longer if volume is large).
6. Aspirate supernatant.
7. Remove tube from magnet and add new media.
8. Insert again for 2 min and repeat 4-5 times.
9. Finally, plate cells in a vitrogen-coated petri dish.

HDEC Media EBM, 15% fetal bovine serum
25 ug/ml cAMP
1 ug/ml hydrocortisone 21-acetate
pen/strep, gentamicin and anti-PPLO

DYNABEADS M-450 COATED WITH GOAT ANTI-MOUSE IgG

(4×10^8 beads/ml (30 mg/ml) in PBS, pH 7.4, containing 0.1% HSA, 0.02% NaN₃: vial contains 10 ml)

1. Resuspend the beads well by vortex.
2. Transfer 500 ul to tube.
3. Add 50 ul of mouse anti-human ELAM-1 (R&D Systems Cat# BBA 16, 1 mg/ml; stock solution)
4. Incubate o/n at 4 °C using end-over-end rotation.
5. Insert in MPC for 2 min, and discard supernatant.
6. Wash 3 times.

7. Resuspend in 1.5 ml of DMEM (1% FBS).
8. Store at 4 °C.