

Preparation of microvascular endothelial cells from tissue (UEA-I coated Dynabeads, Dynabeads CD31 Endothelial Cell)

Materials:

- autoclaved forcipex, scissors
- sterile syringes, needles, scalpells, pincets
- sterile-filters for syringes
- 100µm cell-strainer (Falcon 2360)
- magnetical particle concentrator (MPC)
- Dynabeads coated with UEA-I (see special manual)
- HBSS (Sigma H-2387) containing antibiotics and 0,01M Hepes
- HBSS (Sigma H-2387) containing antibiotics, 0,01M Hepes and 5% SCS
- Trypsin/EDTA solution (Trypsin (Sigma T-8253), 0,3% and EDTA (Merck 1.08418), 1% in HBSS), pH=7,4, sterile filtered
- M199 (Sigma M-5017) containing antibiotics, 20% SCS (=NECM)
- M199 (Sigma M-5017) containing antibiotics, 20% SCS, 5U/ml Heparin (Liquemin, Lovenox, ...), 5µg/ml ECGS (=ECM1) or
- (*optional*: Endothelial Cell Basal Medium (EBM, Promocell C-22210) containing antibiotics, 20% SCS, 5U/ml Heparin (Liquemin, Lovenox, ...), 5µg/ml ECGS (=ECM2))
- Gelatine solution (1% in PBS)
- all solutions prewarmed to 37°C, except HBSS/5%SCS (chilled on ice or to 4°C)
- tissue culture plastic (centrifuge tubes, dishes, flasks, cell-scrappers, ...)

Procedure:

1. Rinse tissue with HBSS
2. Cut tissue into small pieces (approx. 5x5mm)
3. Place pieces into a petri-dish containing 10-20ml Trypsin/EDTA solution
4. Incubate for 45-60 minutes at 37°C
5. Squeeze pieces by using the blunt edge of a scalpel blade and discard pieces
6. Filter solution through a 100µm cell-strainer into a centrifuge tube
7. Centrifuge for 5 minutes at 1500 rpm
8. Discard supernatant
9. Resuspend pellet in 500µl ice-cold HBSS/5%SCS
10. Transfer resuspendet pellet into a 5ml cryo-vial
11. Add 60-70µl coated Dynabeads
12. Incubate 10-15 minutes on ice with agiate shaking
13. Place cryo-vial into the MPC
14. (*Optional*: transfer supernatant to a tissue culture vessel containing NECM for growing non-endothelial cells)
15. Wash Beads/Endothelial cells 3-5 times:
 - a) Add 2,5ml HBSS/5%SCS
 - b) Agiate gently
 - c) Place cryo-vial into the MPC
 - d) Aspirate supernatant
16. Resuspend beads in ECM1 or ECM2
17. Transfer resuspended cells into an appropriate tissue culture vessel, coated with 1% Gelatine

18. Incubate both vessels (EC's and optional NEC's) at 37°C
19. Change medium after 24-48 hours
20. Grow, split and freeze as usual