



Human umbilical vein endothelial cells (HUVECs)

Isolation of HUVECs from umbilical cord

Reagent and instrument requirements

Materials

- 500 mL glass beaker
- Sterile tweezers
- Sterile scalpel
- Sterile 20 mL syringe
- Sterile Gavage Feeding Needles (Fine Science Tools, # 18061-20)
- Sterile clamps (e.g. Carl Roth # N141.1)
- Double Dead Ender Cap, male (Qosina # 65802)
- Sterile 50 mL conical tubes
- 30 μ m nylon mesh or Pre-Separation Filters (# 130-041-407)

Lab equipment

- Centrifuge
- Laminar flow hood (biohazard containment hood)
- Microscope
- Hemocytometer
- Water bath (37 °C)

Reagents

- Phosphate-buffered saline (PBS) (without Ca^{2+} or Mg^{2+})
- PBS/2 mM EDTA
- Trypsin-EDTA solution (0.25%, Sigma-Aldrich, # T4049)
- Trypan blue
- Fetal bovine serum (FBS)

Protocol



1. Clean outer surface of fresh human umbilical cord (approximate length 20 cm) with PBS.



2. To remove the injured end of the cord, or the damage caused by clamping, make a clean cut on the interior side of the injury with a scalpel at a distance of at least 1 cm.

To obtain a clean, straight cut, grip the cord with a pair of sterile tweezers and place the scalpel along the inside line of the tweezers, cut carefully.



3. Feeding needles and Double Dead End Caps needed for the preparation.



4. After making the straight cut, the cord will look like this.

- 1 Artery
- 2 Vein

The umbilical vein can be easily recognized since it is the vessel with the largest diameter.



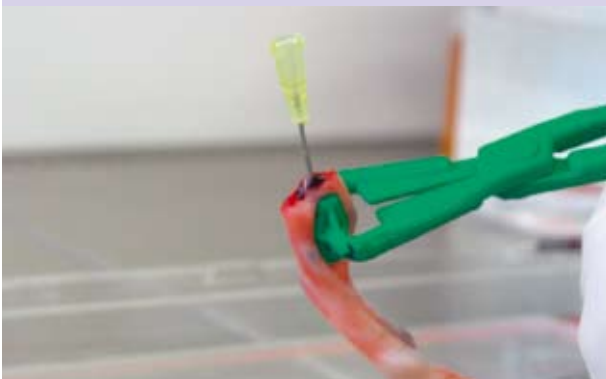
5. At one end, insert a feeding needle carefully into the vein without damaging the surrounding tissue.



6.
Hold it in place with a clamp.



7.
Fill a 20 mL syringe with PBS + 2 mM EDTA and rinse the vein via the feeding needle until no remnants of blood are visible in the eluate.



8.
Insert a second feeding needle into the vein at other end of the cord and hold with forceps.



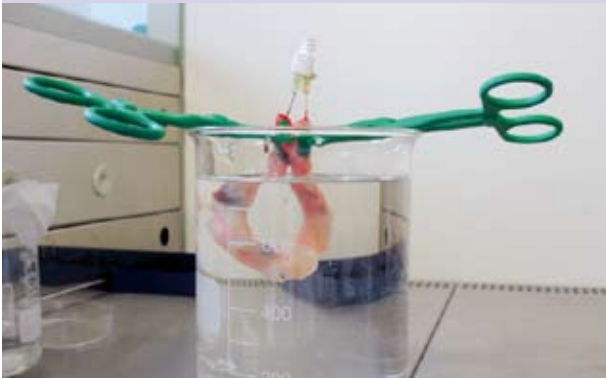
9.
Check for a continuous flow from this end by rinsing the vein once more with PBS + 2 mM EDTA.

10.
Fill a second syringe with 5 mL of a Trypsin-EDTA solution, prewarmed to 37 °C. Without forming air bubbles, replace the first syringe with the second syringe.

11.
Inject 5 mL of the prewarmed Trypsin-EDTA solution.



12. Before injecting another 1–2 mL, seal the opposite end with a Double Dead Ender Cap to prevent collapse of the vein.



13. Suspend the cord in a U-shape in the 500 mL beaker with PBS prewarmed to 37 °C.

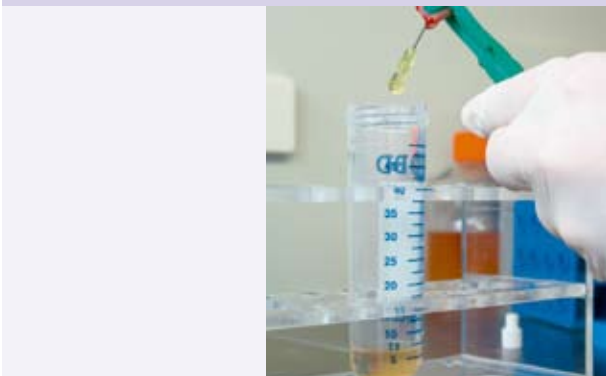
14. Incubate for 15–20 minutes at 37 °C.



15. Place the cord on a soft surface (e.g. tissue paper) and carefully massage for 5 minutes with two fingers.

16. Pipette 2 mL of FBS into a 50 mL conical tube.

17. Fill a syringe with 8 mL of PBS + 2 mM EDTA and attach to one feeding needle without letting air bubbles in.



18. Remove cap from opposite end, carefully flush cord with PBS + 2 mM EDTA and collect all effluent in the conical tube containing the FBS.



19.
The eluate should be cloudy with particles of cellular material.

20.
Pass cells through a 30 µm nylon mesh (Pre-Separation Filters, # 130-041-407) to remove cells clumps.
▲ Note: This step is not required if the cells are directly cultivated without separation.

21.
Centrifuge eluate at 200×g for 10 minutes at room temperature. Aspirate supernatant completely.

22.
Resuspend cells thoroughly in 1 mL of PBS and count viable cells by Trypan blue exclusion in a hemocytometer.

23.
Proceed directly to magnetic labeling and separation, for example:
isolation of CD31⁺ cells using the CD31 MicroBead Kit (# 130-091-935),
isolation of CD105⁺ cells using CD105 MicroBeads (# 130-051-201), or
isolation of CD146⁺ cells using the CD146 MicroBead Kit (# 130-093-596).
For details refer to the respective data sheet.

All protocols and data sheets are available at www.miltenyibiotec.com.



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