# Generation of Human Umbilical Vein Endothelial Cells from Cords

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**[Abstract]** Human umbilical vein endothelial cells (HUVEC) can be isolated from normal human umbilical veins, and are responsive to cytokine stimulation by expressing cell adhesion molecules. These cell systems are commonly used for physiological and pharmacological investigations, such as macromolecule transport, blood coagulation, and angiogenesis. This protocol describes the generation of HUVECs.

## Materials and Reagents

1. Collagenase I (Life Technologies, Invitrogen™, catalog number: 17100-017)
2. Endothelial cell growth supplement (ECGS) (Sigma-Aldrich, catalog number: E2759)
3. M199 medium
4. Fetal calf serum (FCS)
5. Phosphate buffered saline (PBS)
6. Pen/Strep
7. Sodium pyruvate
8. Antibiotic
9. Ethanol
10. Collagenase solution
11. Culture medium (see Recipes)

## Equipment

1. Needle
2. Clamps
3. Syringes
4. Syringe filter
5. Parafilm
6. Heparin
7. Culture hood
8. Sterilized beaker

9. 50 ml tube

## Procedure

1. Wash all tools, glassware and tray in detergent and then put them in ethanol for 20 min under UV in the culture hood.
2. Prewarm PBS and collagenase I (100 ml of PBS for one cord) at 37 °C.
3. Dilute 1 ml of collagenase (20 mg/ml) into 20 ml of warm PBS, spin at 1,000 *x g* for 5 min.
4. Discard the pellet and filter the supernatant using syringe filter under sterile conditions.
5. Take the cord from the bottle, cut ~1 cm from each site (because the ends may be contaminated). Check if the cord is damaged (holes etc), if so discard it.
6. There are three blood vessels in the cord, find the biggest one.
7. Cut the very end of the needle, insert it in the biggest blood vessel hole, fix the needle with the clamp.
8. Screw on the syringe, wash the cord with warm PBS until no blood is leaking from the cord.
9. Put the second clamp at the other end of the cord.
10. Pump 20 ml of collagenase solution into cord, be careful not to blow up the cord.
11. Incubate at 37 °C for 10 min in a sterilized beaker, seal with parafilm.
12. Cut the lower end open. Put all collagenase I to a 50 ml tube, spin at 1,000 *x g* for 5 min.
13. Remove supernatnat, resuspend pellet in 3 ml complete M199 medium.
14. Plate on to a 3 cm tissue culture dish.

## Recipes

1. Culture medium
   20% (v/v) FCS
   10 µg/ml ECGS
   100 U/ml antibiotic
   Sodium pyruvate
   20 U/ml heparin
   All dissolved in M199 medium

## References

1. Jaffe, E. A., Nachman, R. L., Becker, C. G. and Minick, C. R. (1973). Culture of human endothelial cells derived from umbilical veins. Identification by morphologic and immunologic criteria. *J Clin Invest* 52(11): 2745-2756.
2. Ratcliffe, K. E., Tao, Q., Yavuz, B., Stoletov, K. V., Spring, S. C. and Terman, B. I. (2002). Sck is expressed in endothelial cells and participates in vascular endothelial growth factor-induced signaling. *Oncogene* 21(41): 6307-6316.

 