Materials and Equipment

Materials

- Human Umbilical Vein Endothelial Cells (HUVEC) (AC005-F)
- Umbilical Vein Endothelial Cellutions Media (U-GRO)
- TrypLE Express
- 1X PBS Ca^{2+}/Mg^{2+}-free

Equipment

- Incubator, 37°C/ 5% CO_2.
- Tissue culture vessels
- Water Bath, 37 °C
- Centrifuge
- Pipettes
- 15.0 & 50.0 ml tubes

Procedure

1.	Adopt aseptic practices for all steps involving cell culture.
2.	Remove cryovial from shipping container to check that it is still frozen—if thawed contact DV Biologics. Transfer frozen vial to liquid nitrogen until you are ready to thaw and begin cell culture.
3.	Thaw cryovial in 37°C water bath with agitation in FBS (see How to Thaw Cells).
4.	Transfer contents (1 to 2ml) of cryovial to 50ml conical tube. Slowly add dropwise with agitation 9ml of (pre-warmed U-Gro.
5.	Centrifuge 50ml conical tube at 400g for 5 min at 4°C
6.	Remove supernatant; disperse cell pellet with U-GRO; add sufficient U-GRO to make 1 to 5ml; determine cell count (see How to Count Cells)
7.	Seed cells at densities 1.0×10^3 cells/ cm^2 in U-GRO; incubate at 37°C, 5% CO_2; replace spent medium with fresh medium every 2 to 3 days.
8.	When cells are 80 to 90% confluent, they can be subcultured.
9.	To subculture, remove spent medium; rinse cells twice with PBS (pre-warmed at 37°C); dissociate cells with trypsin solution (37°C, 5 to 10min); quench dissociation with U-GRO; centrifuge cells at 400g for 5 min at 4°C. (for details see How to Passage Cells)
10.	Remove supernatant; disperse cell pellet with U-GRO; add sufficient U-GRO to make 1 to 5ml; determine cell count.
11.	Seed cells at densities 3.0×10^3 cells/cm^2 in U-GRO; incubate at 37°C, 5% CO_2; replace spent medium with fresh medium every 2 to 3 days or alternatively freeze cells. To freeze cells see How to Freeze Cells.