**GFP Expressing Human Umbilical Vein Endothelial Cells (GFP HUVECs)**

**Catalog #:** GF01  
**Cell #:** 5x10^5 cells in frozen vial  
**Storage:** 37°C CO₂ incubator or Liquid Nitrogen  
**Product Format:** Frozen

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**General Information**

GFP expressing human umbilical vein endothelial cells were isolated from normal human umbilical vein and transfected with GFP-lentiviral particles at passage 1. Puromycin resistant GFP-HUVECs are passage 3 and are shipped frozen. **ENDO-Growth Medium containing 5% serum and growth supplements** are recommended for culture. Cells have an average additional population doubling levels >18 when cultured.

**Characterization of the cells**

- Cytoplasmic VWF / Factor VIII: >95% positive by immunofluorescence
- Cytoplasmic uptake of Di-I-Ac-LDL: >95% positive by immunofluorescence
- Cytoplasmic PECAM1: >95% positive by immunofluorescence

HUVECs are negative for HIV-1, HBV, HCV, and mycoplasma.

**Recommended Products**

- **ENDO-Growth Media – MED001**  
  - Contains 475 ml of ENDO-Basal Media and 25 ml of ENDO-Growth Supplement combined. Which is freshly prepared for your convenience
  
- **ENDO-Growth Kit – EGK001**  
  - Contains 475 ml of ENDO-Basal Media and 25 ml of ENDO-Growth Supplement in separately to be mixed to make growth media

- **Smooth Coat Solution – SC300**  
  - Bio compatible complex of extracellular matrix binding solution

- **AlphaBioCoat Solution – AC001**  

- **Cell Detachment Solution – ADF001**  
  - Contains protease and collagenase activities in an isotonic, phosphate buffer solution with EDTA to detach primary cells and cell lines

- **1X Phosphate Buffer Solution - PBS300**

**Shipping**

Frozen cells are shipped on dry ice.

**Handling of Arriving Cells**

When you receive the cells, store in liquid nitrogen to keep the cells frozen or thaw cells according to the protocol for culture.

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SUBCULTURE PROTOCOL

1. Coating T25 flasks:
   a. Add 2 ml AlphaBioCoat Solution (AC001) into a T25 flask and ensure entire interior surface is coated with solution. After 30 minutes, dispose of Smooth Coat Solution by aspiration. Gently rinse and aspirate flask with phosphate buffer solution (PBS300). The flask is now ready for use (no need for overnight incubation when coated with AC001).
   b. If you are using the coated flask the same day, add about 4 ml of Endo-Growth media (MED001) to the coated flask. If the media changes color from pink to yellow, aspirate and discard the media. Add 4 ml of fresh media to the coated flask.

2. Thaw the cells in a 37°C water bath. Once you see a small amount of ice left in the vial, spray the vial with 70% Ethanol and wipe it down.

3. Transfer the vial into your Biosafety cabinet.

4. Using a 2 or 5 ml pipet, pipet the cells out of the vial.

5. Transfer your cell suspension in to your coated flask (which contains 4 ml media).

6. You should have a total working volume of 5 ml of cell suspension in the flask; close the cap. Make sure cells are evenly distributer in the flask by moving the flask left and right five times. Move it up and down for and additional five times.

7. Place flask in a 37°C incubator with 5% CO2. If flask is not vented, please loosen cap.

8. Change media after 48 hours.

9. Place flask in 37°C incubator until cells are at 90% confluence. Change media every 2 days.

10. When flask is at 90% confluence, aspirate media from flask.

11. Rinse T25 flask containing cells with 5 ml 1XPBS (PBS300).

12. Gently aspirate out the PBS after rinsing, and discard.

13. Add 2 ml of RT trypsin/EDTA or Cell Detachment Solution (ADF001) to T25 flask containing cells (ensure entire interior surface is cover).

14. Place T25 flask containing cells into 37°C incubator for 1 or 2 minutes (cells will normally come off of the surface within 1 or 2 minutes).

15. Suspend the cells with 15 ml of ENDO-Growth medium (MED001) and transfer equally into 3 pre-coated T25 flasks (the cells are now at a subculture ratio of 1:3).

16. There is no need to spin cells during subculture.

17. Proliferating cell culture: ENDO-Growth medium (MED001) should be changed every 2 days. The cells normally become confluent within 7 days (when split at a 1:3 ratio).

18. Use ENDO- Basal media (MED002) containing 0.5% FBS to induce quiescent cells (after 18-24 hours).

Note: Should any issues arise while using our cells, our team is here to help troubleshoot any issues. Our cells are backed by our one-time replacement or refund policy. Our recommended protocol including recommended products must be used to be eligible for replacement or refund. Cells that have been refrozen are no longer eligible for refund or replacement.
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