NEUROMICS

Human Glomerular Microvascular Endothelial Cells

Catalog #: HEC04

Cell #: > $5X10^5$ cells

Storage: 37°C CO_2 incubator or Liquid Nitrogen

Product Format: Frozen or Proliferating in culture

General Information

Human Glomerular Microvascular Endothelial Cells were isolated from normal human kidney glomeruli tissue. Passage 1, cells are ship on dry ice in a vial or in proliferating culture with a confluence of >90%. ENDO-Growth Media is recommended for culture. Cells have an average additional population doubling levels >15 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

HLIMECs 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

OR

- ENDO-Growth Kit – EGK001
  - Contains 475 of ENDO-Basal Media and 25 ml of ENDO-Growth Supplement in separately to be mixed to make growth media

- Smooth Coat Solution – SC300
  - Biocompatible complex of extracellular matrix binding solution

OR

- AlphaBioCoat Solution – AC001
  - Premium Smooth Coat Solution. Biocompatible complex of extracellular matrix binding solution with growth factors. Ideal for culturing cells from frozen.

- 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

Shipped on dry ice frozen in a vial or proliferating in culture in T25 flask.

Handling of Arriving Cells

If you received the cells froze: store in liquid nitrogen to keep the cells frozen or thaw cells according to the protocol for culture.

If you receive the cells proliferating in culture: leave the flask in 37°C CO_2 incubator for 1 hour first, and then replace the transport medium with fresh Full medium. Let the cells grow for 24 hours before subculture

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Phone 866-350-1500 • fax 612-677-3976 • email: pshuster@neuromics.com

Note: Handling human derived products is potentially bioharzadous. Although each cell strain tests negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions must be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working these materials. Never mouth pipette. We recommend followinfg the universal procedures for handling products of human origin as the minimum precaution against contamination.

SUBCULTURE PROTOCOL (From Frozen)

Note: If you have any questions or need clarification regarding the protocol for culturing these cells, please reach out to Dr. Jensen Auguste at (978) 608-1766 with your questions before beginning.

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 AlphaBioCoat 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 to the coated flask. *If the media changes color from pink to yellow, aspirate and discard the media. Add 4ml 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 vail, spray the vail with 70% Ethanol and wipe it down.
3. Transfer the vail into your Biosafety cabinet.
4. Using a 2 or 5ml 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 5ml 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% C02. 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 (cat#PBS300).
12. Gently aspirate out the PBS after rinsing, and discard.
13. Add 2ml of RT trypsin/ EDTA or Cell Detachement 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 15ml 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).

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SUBCULTURE PROTOCOL (Proliferating in Culture)

Note: If you have any questions or need clarification regarding the protocol for culturing these cells, please reach out to Dr. Jensen Auguste at (978) 608-1766 with your questions before beginning.

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 4ml of fresh media to the coated flask.

2. Inspect to the confluence of the flask. If the flask is not 90% confluence, remove transport media and add 5ml of fresh media to the flask. Place flask in 37°C incubator until cells are at 90% confluence. Change media every 2 days.

3. If flask is at 90% confluence, aspirate transport media from flask

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

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

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

7. 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).

8. Suspend the cells with 15ml 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).

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

10. 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)

11. 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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