Human Brain Microvascular Pericytes (HBMPVCs)

Catalog #: HMP104
Cell #: > 5x10^6 cells in frozen vial

Storage: 37°C CO₂ incubator or Liquid Nitrogen
Product Format: Frozen

General Information
Human Brain Microvascular Pericytes were isolated from normal human brain cortical tissue. Puromycin resistance HBMVPCs are shipped at passage 3 on dry ice in a vial. Pericyte-Growth medium containing 5% fetal bovine serum and growth supplement is recommended for culture. Cells have an average additional population doubling levels >12 when cultured.

Characterization of the cells
- Cytoplasmic VWF / Factor VIII: <2% positive by immunofluorescence
- Cytoplasmic uptake of Di-L-Ac-LDL: <10% positive by immunofluorescence
- Cytoplasmic Alpha-Actinin Filaments: >80% positive by immunofluorescence
- Cytoplasmic Desmin Intermediate Filaments: >80% positive by immunofluorescence

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

Recommended Products
- Pericyte Growth Media
  - Growth Media designed for pericyte growth. Contains the following two components: 495 ml of DMEM (high glucose) and 5 ml of Pericyte-Growth supplement.
- Smooth Coat Solution – SC300
  - Biocompatible complex of extracellular matrix binding solution
OR
- 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
Shipped on dry ice frozen in a vial.

Handling of Arriving Cells
Store in liquid nitrogen to keep the cells frozen or thaw cells according to the protocol for culture.

Note: Handling human derived products is potentially biohazardous. 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 following the universal procedures for handling products of human origin as the minimum precaution against contamination.

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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 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 Pericyte-Growth media 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 the 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 distributed 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 (cat#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 10 ml of Pericyte-Growth medium (PGB001) and transfer equally into 2 pre-coated T25 flasks (the cells are now at a subculture ratio of 1:2).
   a. Future passages can have a subculture ratio of 1:3, once the cells are more stable.
      i. Suspend the cells with 15 ml of Pericyte-Growth medium and transfer equally into 3 pre-coated T25 flasks

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

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

18. Use Pericyte-Basal media (PGB002) containing 0.5% FBS to induce quiescent cells (after 18-24 hours).

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