NEUROMICS

Human Retinal Microvascular Pericytes (HRMPVCs)

Catalog #: HMP106

Cell #: > $5X10^5$ cells (>90% confluent in T25 flask)

Storage: 37°C CO_2 incubator or Liquid Nitrogen

Product Format: Frozen or proliferating in culture

General Information

Human Retinal Microvascular Perictyes were isolated from normal human retinal tissue. HRMVPCs are shipped at passage 3 on dry ice in a vial or with a confluence of >90% (catalog HMP106-T25). 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.

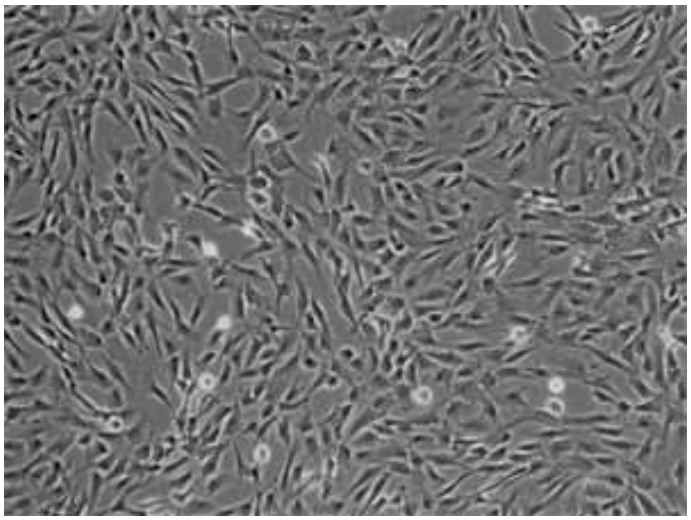

Image: Retinal Pericytes in culture

Characterization of the cells

- Cytoplasmic VWF / Factor VIII: <2% positive by immunofluorescence
- Cytoplasmic uptake of Di-I-Ac-LDL: <2% positive by immunofluorescence
- Cytoplasmic Alpha-Actinin Filaments: >80% positive by immunofluorescence
- Cytoplasmic Desmin Intermediate Filaments: >80% positive by immunofluorsesnce

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

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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
  - 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.

Handling of Arriving Cells

Store in liquid nitrogen to keep the cells frozen or thaw cells according to the protocol for culture. For cells arriving in a T25 flask (cat HMP106-T25) leave the cells in 37°C CO_2 incubator for 1 hour. Then, replace transport medium with fresh Pericyte-Growth medium. Let grow from 24 hours before subculture.

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.

SUBCULTURE PROTOCOL - 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 Pericyte-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 the 4 ml media).

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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 10ml 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).

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 1:2 ratio)

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

SUBCULTURE PROTOCOL - T25 flask (catalog HMP106-T25)

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. Add 2 ml Smooth Coat Solution (SC300) into 3- 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 SC300). Add fresh media to flask, if color changes from pink to yellow, discard the media, and add fresh media to each flask.

2. Inspect to make sure Flask is at 90% confluence, if not 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 (cat#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).

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8. Suspend the cells with 10ml 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).

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

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

11. Use Pericyte- Basal media (PGB002) 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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