ANGIO-PROTEOMIE

DATA SHEET

**Mouse Pulmonary Microvascular Endothelial Cells**

**Order Information**

**Product Name:** Mouse Pulmonary Microvascular Endothelial Cells (mPulMENCs)
**Catalogue Number:** **cAP-m0003**
**Product Format:** Frozen vials
**Cell Number:** > 5 x $10^5$cells/vial

**General Information**

mPulMENCs (cAP-m0003) are pooled cells isolated from peripheral tissues of pulmonary lobes of C57BL6 mice. mPulMENCs are shipped in proliferating culture with >90 confluence (the cells are provided @ passage 2). ENDO-Growth medium (contains 5% serum and growth supplements, Cat#cAP-02) is recommended for cell culture and these cells have a guaranteed average additional population doubling levels 6-8 when cultured following the detailed protocol described below).

**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
**mPulMENCs** are negative for bacteria, yeast, fungi, and mycoplasma**.**

**Product Use:** mBMECs are for research use only.

**Shipping:** Frozen vials in dry ice package

**Handling of Arriving Cells**

When you receive the cells in a frozen vial, you can transfer the vial of cells into a -80ºC freezer for short period storage or a liquid nitrogen tank for long term storage. Thaw the cells in a 37°C water bath, and then transfer the cells in a T25 flask pre-coated with Quick coating solution (cAP-01) as described in details in Subculture Protocol.

**Note: For certain endothelial cells, fibronectin (cAP-42) coated culture wares are essential (check cAP-42 for detailed protocols).**

**Subculture Protocol**

A) Pre-coating of T25 flasks: Add 2ml of Quick Coating Solution (**cAP-01**) into one T25 flask and make sure whole surface of the flask is covered with the coating solution. Five minutes later, dispose excessive Quick Coating Solution by aspiration and the flask is ready to be used (no need for overnight incubation when using Quick Coating Solution). Other extracellular matrix can be used including gelatin, collagen, and fibronectin and you are advised to test the conditions for using those materials in advance.
B) Rinse the cells in T25 flask with 5ml HBSS (**Room Temperature, RT**) twice.
C) Add 2ml of Trypsin/EDTA (**RT**) (cAP-23) into one T25 flask (make sure the whole surface of the T25 flask is covered with Trypsin/EDTA), and gently dispose the excessive Trypsin/EDTA solution **within 20 seconds** with aspiration.
D) Leave the T25 flask with the cells at **RT** for 1 minute (the cells usually will detach from the surface within 1-2 minutes). You can monitor the cells under microscope and when most of cells become rounded up, hit the flask against the bench surface, and the cells will move on the surface of the flask when monitoring under microscope.
E) Add 5ml Trypsin Neutralization Buffer and spin the cells down with 800g for 5 minutes.
F) Re-suspend the cell pellet with 10 - 15ml of EGM full medium and the cell suspension is transferred directly into 2 or 3 pre-coated T25 flasks (5ml each, and the cells are sub-cultured at 1:2 to 1: 3 ratios)
G) Change medium every 2-3 days and cells usually become confluent within 7 days.
H) If you need prepare quiescent cells, when cells are almost confluent, replace EGM full medium with Endothelial Basal Medium (EBM, cAP-03) containing 0.5% FBS about 8-12 hours before your experiments.

**Related Products:**

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| Quick Coating Solution | cAP-01 | 240ml | Angio-Proteomie |
| Endothelial Growth Medium | cAP-02 | 500ml | Angio-Proteomie |
| Endothelial Basal Medium | cAP-03 | 500ml | Angio-Proteomie |
| HBSS w/o $Ca^{2+}$ , $Mg^{2+}$ | cAP-11 | 100ml | Angio-Proteomie |
| Cell Freezing Solution (FBS) | cAP-22 | 50ml | Angio-Proteomie |
| Cell Freezing Solution (Non-FBS) | cAP-22B | 50ml | Angio-Proteomie |
| Trypsin/EDTA Solution | cAP-23 | 100ml | Angio-Proteomie |
| Trypsin Neutralization Solution | cAP-28 | 100ml | Angio-Proteomie |
| ITS (100x) | cAP-26 | 10ml | Angio-Proteomie |
| L-Glutamine-MAXIMUM (100x) | cAP-27 | 100ml | Angio-Proteomie |
| Human Plasma Fibronectin Solution | cAP-42 | 1mg/ml | Angio-Proteomie |



11 Park Drive, Boston, MA 02215, USA | Tel: 617-549-2665 | Fax: 480- 247-4337 | E-mail: angioproteomie@gmail.com