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## RFP Expressing Human Retinal Microvascular Pericytes

### ORDER INFORMATION

**Name of Cells:** RFP Expressing Human Retinal Microvascular Pericytes Cells (RFP-HRMPCs)  
**Catalogue Number:** cAP-0025RFP  
**Product Format:** Proliferating culture  
**Cell Number:** > 90% confluent (> 3 x 10<sup>5</sup> cells) in T25 flask

### General Information

HRMPCs (cAP-0025) are initiated by elutriation from dissociated normal human retinal tissue and transfected with RFP-Lentiviral particles at passage 2. Zeocin resistant RFP-HRMPCs (cAP-0025RFP) were selected and shipped in proliferating culture with >90 confluence (the cells are provided @ passage 4-5). Pericytes growth medium (cAP-09, containing FBS and growth factor supplements) is recommended for cell culture and these cells have an average population doubling capacity > 8 when cultured following the detailed protocol described below).

### Characterization of the cells

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

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

### Product Use

RFP-HRMPCs are for research use only.

### Shipping status

Proliferating cells in T25 flask.

### Handling of Arriving Cells

When you receive the cells, leave the flask in 37°C CO<sub>2</sub> incubator for 1 hour first, and then replace the transport medium with fresh Pericyte growth medium (cAP-09, containing FBS and growth factor supplements). Let the cells grow for 24 hours before subculture.

### 1. Subculture Protocol:

A) Coating T25 flasks: Add 2ml 0.1% Quick Coating Solution (cAP-01) into one T25 flask and make sure whole surface of the flask is covered with the coating solution.

Contact & Ordering Information: Angio-Proteomie, 11 Park Drive, Suite 12, Boston, MA 02215, USA. Fax: (480) 247-4337, [angioproteomie@gmail.com](mailto:angioproteomie@gmail.com)

Five minutes later, dispose Quick Coating Solution by aspiration and the flask is ready to be used (no need for overnight incubation when coated with Quick Coating Solution).

- B) Rinse the cells in T25 flask with 5ml PBS (**Room Temperature, RT**) twice.
- C) Add 2ml of Trypsin/EDTA (**RT**) (Invitrogen Catalogue number: 25300-062) into T25 flask (make sure the whole surface of the T25 flask is covered with Trypsin/EDTA), and gently dispose the Trypsin/EDTA solution **within 10 seconds** with aspiration.
- D) Leave the T25 flask with the cells at **RT** for 1 minute (the cells will normally come off the surface within 1 minute).
- E) Suspend the cells with 20ml of Pericytes medium (contains FBS and growth factor supplement) and the cell suspension is transferred directly into 2x pre-coated T25 flasks (10ml each, and the cells are subcultured at 1:2 ratio)

**(Note: No need spin the cells during the subculture process).**

## 2. Cell culture protocol (proliferating):

- A) Culture medium: Pericyte growth medium should be changed every 2 days.
- B) The cells normally become confluent within 7 days (when split at 1:2).

## 3. Preparation of quiescent cells:

- A) Pericyte basal medium (with 0.5% FBS) is used to induce quiescent pericytes (after 10-15hours).

Other products needed:

Items	Company	Cat #
Quick Coating Solution	Angio-Proteomie	cAP-01
Pericyte Growth medium	Angio-Proteomie	cAP-09
Pericyte Basal medium	Angio-Proteomie	cAP-12
PBS	Invitrogen	10010
Trypsin/EDTA	Invitrogen	25300-062

**Caution:** Handling human derived products is potentially biohazardous. Although each cell strain testes 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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