



11 Park Drive, Suite 12
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RFP Expressing Human Retinal Microvascular Endothelial Cells

ORDER INFORMATION

Name of Cells: RFP Expressing Human Retinal Microvascular Endothelial Cells (RFP-HRMECs)
Catalogue Number: cAP-0010RFP
Product Format: Proliferating culture
Cell Number: > 90% confluent (>5x10⁵ cells) in T25 flask

General Information

HRMECs (cAP-0010) are initiated by elutriation from dissociated normal human retinal tissue and transfected with RFP-Lentiviral particles at passage one. Zeocin resistant RFP-HRMECs (CAP-0010RFP) were selected and shipped in proliferating culture with >90 confluence (the cells are provided @ passage 4-5). ENDO-Growth medium (contains 5% serum and growth supplements, Cat# cAP-02) is recommended for cell culture and these cells have an average population doubling capacity > **16** 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

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

Product Use

RFP-HRMECs 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 CO₂ incubator for 1 hour first, and then replace the transport medium with fresh ENDO-Growth medium. 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. Five minutes later, dispose Quick Coating Solution by aspiration and the flask is

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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 Trpsin/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 ENDO-Growth medium and the cell suspension is transferred directly into 4 x pre-coated T25 flasks (5ml each, and the cells are subcultured at 1:4 ratio)

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

2. Cell culture protocol (proliferating):

- A) Culture medium (ENDO-Growth medium) is changed every 2 days.
- B) The cells normally become confluent within 7 days (when split at a 1:4 ratio).

3. Preparation of quiescent cells:

- A) ENDO-Basal medium (cAP-03) containing 0.5% FBS is used to induce quiescent endothelial cells (after 18-24hours).

Other products needed:

Items	Company	Cat #
Quick Coating Solution	Angio-Proteomie	cAP-01
ENDO-Growth medium	Angio-Proteomie	cAP-02
ENDO-Basal medium	Angio-Proteomie	cAP-03
ENDO-Growth Supplement	Angio-Proteomie	cAP-04
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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