

**RFP Expressing hTert Immortalized Human Retinal Microvascular Pericytes**
**ORDER INFORMATION**

**Name of Cells:** RFP Expressing hTert Immortalized Human Retinal Microvascular Pericytes Cells (**RFP-hTert-HRMVPCs**)  
**Catalogue Number:** **cAP-0025hTert-RFP**  
**Product Format:** Frozen Vial  
**Cell Number:** > 5 x 10<sup>5</sup>/vial

**General Information**

HRMVPCs (**cAP-0025**) are initiated by elutriation from dissociated normal human retinal tissue. Zeocin resistant **RFP-hTert-HRMVPCs** are selected from HRMVPCs after infected with lentiviruses expressing RFP and hTert. The cells are shipped in frozen vials (the cells are provided @ passage 4). Pericyte Growth Medium (cAP-09, containing FBS and growth factor supplements) is recommended for cell culture and these cells have a minimum of population doubling capacity > **30** (tested so far) 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  
 hTert-HRMVPCs are negative for HIV-1, HBV, HCV, and mycoplasma.

**Product Use:** hTert-HRMVPCs are for **Research Use Only**.

**Shipping status:** frozen vials.

**Handling of Arriving Cells**

When you receive the cells in a frozen vial, you can transfer the vial of cells into a -80°C 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.

**1. 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 10-15 ml of PGM 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 or 1:3 ratios)
- G) Change medium every 2-3 days and cells usually become confluent within 7 days (when split at a 1:2 ratio).

**Related products**

|                                                          |         |        |                 |
|----------------------------------------------------------|---------|--------|-----------------|
| Quick Coating Solution                                   | cAP-01  | 240ml  | Angio-Proteomie |
| Pericyte Growth Medium                                   | cAP-09  | 500ml  | Angio-Proteomie |
| Pericyte Growth Medium ( <b>super rich formulation</b> ) | cAP-09B | 500ml  | Angio-Proteomie |
| Pericyte Basal Medium                                    | cAP-09C | 500ml  | Angio-Proteomie |
| HBSS w/o Ca <sup>2+</sup> , Mg <sup>2+</sup>             | 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 |

**Caution:** Handling human tissue derived products is potentially bio-hazardous. Although each cell strain is tested 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.