

RFP-Human Induced Pluripotent Stem (IPS) Cells-Derived Endothelial Precursor Cells**ORDER INFORMATION**

Name of Cells: RFP-Human iPSC-derived Endothelial Precursor Cells
Catalogue Number: **cAP-0500-002-02RFP**
Product Format: Cells in Frozen Vials
Cell Number: > 5 x 10⁵ Cells/vial

General Information**Description**

cAP-0500-002 parental fibroblasts were obtained from a healthy donor. The fibroblasts were reprogrammed by the expression of OCT4, SOX2, KLF4 and MYC gene sequences using Sendai viral transduction. This cell line provides a unique model system for better understanding cell development and differentiation, as well as source material for the development of iPSC derived cells. cAP-0500-002-02 were selected from cAP-0500-002 cells after cultured in Human Endothelial Growth Medium-Serum Free Formulation 2 (EGM-SF2, cAP-02-SF2) for 7 days, with a two steps purification with (1) CD144 and (2) VEGFR2. Cells are supplied at passage 2 after VEGFR2 purification. cAP-0500-002-02RFP cells were selected from cAP-0500-002-02 cells using puromycin after infected with RFP expressing lentiviruses.

SAFETY PRECAUTION

We highly recommend that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

Handling of Arriving Frozen 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.

1. Preparation for Culture***Fibronectin Coated Flasks:***

1. Thaw Fibronectin and make it into final Concentration of 20ug/ml with PBS (1mg into 50ml 1 x PBS).
2. Add sufficient the diluted Fibronectin into culture Flasks (2ml for T25, 3-4ml for T75) and incubated the flasks at 37C for at least 1 hour (preferred overnight).
3. Shortly before add the cell suspension, aspirate the excessive fibronectin and the flask is ready to be used.

Culture Medium:

4. Human Endothelial Growth Medium-Serum Free Formulation 2 (EGM-SF2, cAP-02-SF2) is recommended for passage/culture the Human iPSC-derived Endothelial Precursor Cells.

2. Thaw the Cells

Thaw the cells in a 37°C water bath, and then transfer the cells into a 15ml falcon tube with 10ml EGM-SF2 medium, and spin down the cells with 1000rpm for 8 mins. After resuspend the cells in 5 ml of the EGM-SF2 medium, transfer the cells into a T25 flask pre-coated with Human Fibronectin (cAP-42) as described above. The cells should be cultured at 37C with 5%CO₂ humidified incubator. The cells should be confluent within two or three days.

3. Subculture of the Cells:

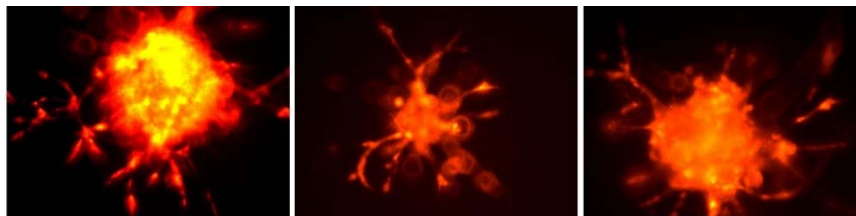
1. Once the cells are confluent, wash the cells twice with PBS (without Ca²⁺ and Mg²⁺) twice and then add 2ml of Trypsin/EDTA (cAP-23); as soon as the cells become rounded, add 10ml trypsin neutralization solution and spin down the cells with 1000rpm for 8min.
2. Resuspend the cells in 15ml EGM-SF2 and add 5ml each into fibronectin coated T25 flasks. The cells should be cultured at 37C with 5%CO₂ humidified incubator, medium changes every two days.

4. Cryopreservation

1. When the cells become confluent, collect the cells the same way as cell subculture Step 1;
2. Resuspend the cells in 2 ml cell culture freezing medium (cAP-22B).
3. Gently resuspend the pellet by pipetting up and down **2 to 3** times with a 1 mL tip, and transfer 1 mL each of the cell suspension into 2 labeled cryovials (Cells from one T25 flask into two vials).
5. Freeze the cells gradually at a rate of 1 C/min until the temperature reaches -70 C to 80 C.
6. The cells should be left at 80C for no more than 24 to 48 hours. Once at 80C, frozen cryovials should be transferred to the vapor phase of liquid nitrogen for long-term storage.

5. Tube Formation Assay (Matrigel Based):

1. Thaw Matrigel (Corning: 354230) Please following manufacturer's instruction.
2. Add 80-100ul of thawed Matrigel into one well of the 96-well plate.
3. Incubate the Matrigel at 37C for at least 30mins until the Matrigel is solidified.
4. Prepare the cell suspension at a density of 2.5x 10⁵/ml cells in EGM-SF2 medium
5. Add 50ul, 100ul, and 150ul of the cell suspension into one Matrigel well.
6. Monitor the cells daily and change the medium every two days with care not to disturb the cells too much.



Angiogenic Sprouting of RFP-Human iPSC-derived Endothelial Precursor Cells on the Surface of Matrigel

Related products

Human Endothelial Growth Medium-Serum Free Formulation 2 (EGM-SF2)	cAP-02-SF2	500ml	Angio-Proteomie
cAP-42 Human Plasma Fibronectin Solution (HPF)	cAP-42	50ml	Angio-Proteomie
Trypsin-EDTA Solution	cAP-23	50ml	Angio-Proteomie
Trypsin Neutralization Solution	cAP-28	1 ml	Angio-Proteomie
Freezing Medium	cAP-53	50ml	Angio-Proteomie
Cell Culture Freezing Medium (NON-FBS)	cAP-53	50ml	Angio-Proteomie

Use Restrictions

These cells are distributed for research purposes only.

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

Warranty

The viability of our products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. We list the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the our recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, our warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans. While we use reasonable efforts to include accurate and up to date information on this product sheet, we make no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. We do not warrant that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. We are not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, we are not liable for damages arising from the misidentification or misrepresentation of cultures.

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.