Angio-Proteomie
A Functional Antibody Supplier

11 Park Drive, Suite 12
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Rat Pulmonary Microvascular Endothelial Cells

ORDER INFORMATION

Name of Cells: Rat Pulmonary Microvascular Endothelial Cells **(RPulMVECs)**
Catalogue Number: **cAP-r0004**
Product Format: Proliferating in flasks or Frozen Vials
Cell Number: > 90% confluent in T25 flask or >5 x 10^5 cells/vial

General Information:

RPulMVECs (**cAP-r0004**) are isolated from lung tissues of adult SD rats. The cells are shipped in proliferating culture with >90 confluence (the cells are provided @ passage 3) or in a frozen vial. Rat Endothelial Growth Medium (REGM, cAP-02r) is recommended for the expansion of RAECs and these cells can be propagated to sixth passage and beyond without losing their morphologic and phenotypic characteristics when cultured following the detailed protocol described below).

Characterization of the cells

PECAM1: **>95% positive by immunofluorescence**
VE-Cadherin: **>95% positive by immunofluorescence**
RPulMVECs are tested negative for mycoplasma.

Product Use: RPulMVECs are for research use only.

Shipping: Proliferating culture in T25 flasks or in frozen vials.

Handling of Arriving Cells

When you receive the cells in a T25 flask, leave the T25 flask in 37°C CO2 incubator for 1 hour first, and then replace the transport medium with fresh REGM full medium. Let the cells grow for 24 hour before subculture.

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 Collagen (Type I) coating solution (cAP-30) as described in details in Subculture Protocol.

Subculture Protocol

A) Pre-coating of T25 flasks: Add 2ml of Collagen (Type I) Coating Solution (**cAP-30**) into one T25 flask and make sure whole surface of the flask is covered with the

coating solution. Leave the T25 in 37°C for a minimum 60minute and then dispose excessive Collagen (Type I) Coating Solution by aspiration and rinse the flask with 5 ml PBS before the flask is ready to be used. Other extracellular matrix can be used including gelatin, and fibronectin, but customers are strongly advised to test the conditions before 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 REGM full medium and the cell suspension is transferred directly into 2 to 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 (when split at a 1:3 ratio).

H) If you need prepare quiescent cells, when cells are almost confluent, replace REGM full medium with Endothelial Basal Medium (EBM, cAP-03) containing 0.5% FBS about 8-12 hours before your experiments.

Related products

Collagen (Type I) Coating Solution	cAP-01	240ml	Angio-Proteomie
Rat Endothelial Growth Medium (REGM)	cAP-02r	500ml	Angio-Proteomie
Endothelial Basal Medium	cAP-03	500ml	Angio-Proteomie
HBSS w/o Ca^{2+} , Mg^{2+}	cAP-11	100ml	Angio-Proteomie
Trypsin/EDTA Solution	cAP-23	100ml	Angio-Proteomie
Trypsin Neutralization Solution	cAP-28	100ml	Angio-Proteomie

Caution: Although primary cells are tested pathogen-free, investigators should handle these cells with caution and treat all animal cells as potential pathogens, since no test procedure can completely guarantee the absence of infectious agents.

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