Angio-Proteomie
A Functional Antibody Supplier

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Bovine Retinal Microvascular Endothelial Cells

ORDER INFORMATION

Name of Cells: Bovine Retinal Microvascular Endothelial Cells **(BReMVECs)**
Catalogue Number: **cAP-b0004**
Product Format: Proliferating cells in T25 or a Frozen Vial
Cell Number: > 90% confluent in T25 flask or Frozen Vial (>5x 10^5/vial)

General Information:

BReMVECs (**cAP-b0004**) are isolated from young healthy bovine retina tissue. The cells are shipped in proliferating culture with >90 confluence (the cells are provided @ passage 2). Endothelial Growth Medium (cAP-02) is recommended for the expansion of **BReMVECs** and these cells can be propagated to at least 10 population doubling times 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**
BReMVECs are tested negative for mycoplasma.

Product Use: bReMVECs are for **Research Use Only**.

Shipping: Proliferating culture in T25 flask or Proliferating cells in T25 or a Frozen Vial.

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 EGM 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) or Quick coating solution (cAP-01) as described in details in Subculture Protocol.

Subculture Protocol

A) Pre-coating of T25 flasks:

(1) Add 2 ml of Collagen Type I coating solution (cAP-30) into one T25 flask and sure that the whole surface of the flask is covered with the coating solution. **Leave the flask in a 37C CO2 incubator for overnight.** The next day, dispose excessive coating solution by aspiration and wash the flask with 5 ml of PBS twice before using.

Or (2) Add 2ml of Quick Coating Solution (**cAP-01**) into one T25 flask and make sure that the whole surface of the flask is covered with the coating solution. **Leave the flask in a 37C CO2 incubator for overnight.** The next day, dispose excessive Quick Coating Solution by aspiration and the flask is ready to be used.

Other extracellular matrix can also be used, but you are advised to test the conditions for using those materials in advance.

B) Rinse the cells in T25 flask with 5ml HBSS w/o Ca^{2+} and Mg^{2+} (**cAP-11**) twice (room temperature, RT).

C) **Add 1.6ml of HBSS w/o Ca^{2+} and Mg^{2+} and 0.4ml of Trypsin/EDTA (RT) (cAP-23) into one T25 flask (make sure the whole surface of the T25 flask is covered with diluted (1:4) Trypsin/EDTA; Monitor the cells under microscope (gently hit the flask with your palm if necessary to help the detachment of the cells) and as soon as the cells come off the surface, transfer the cell suspension into 10ml Trypsin Neutralization Buffer (cAP-28) or full EGM. If there are still cells attached to the surface after two minutes, after collecting the initial 2ml of cell suspension; repeat the step C) one more time (add 1.6ml of HBSS w/o Ca^{2+} and Mg^{2+} and 0.4ml Trypsin/EDTA into the flask and collect the rest of cells in 2 minutes as previously do).**

D) Spin the cells down with **800rpm** for 5 minutes.

E) Re-suspend the cell pellet gently with 14ml of EGM full medium and the cell suspension is transferred directly into 2 pre-coated T25 flasks (7ml each, and the cells are sub-cultured at 1:2). **Leave the flasks in a 37C CO2 incubator and try not to disturb the cells in the initial 12 hours (You may see some floating cells the next daya fter splitting the cells; this is common for Bovine MV endothelial cells).**

F) Change medium every other day and cells usually become confluent within 5-7 days (when split at a 1: 2 ratio).

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

Related products

Quick Coating Solution	**cAP-01**	**240ml**	**Angio-Proteomie**
Endothelial Growth Medium	**cAP-02**	**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**

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