11 Park Drive, Suite 12
Boston, MA 02215

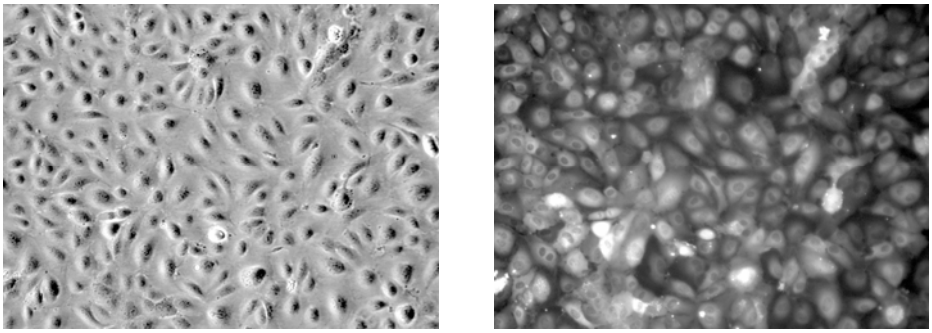
GFP-Expressing Human Brain Microvascular Endothelial Cells

ORDER INFORMATION

Name of Cells: GFP Expressing Human Brain Microvascular Endothelial Cells **(GFP-hBMEnds)**
Catalogue Number: **cAP-0002GFP**
Product Format: Proliferating culture
Cell Number: > 90% confluent in T25 flask

General Information

GFP-hBMEnds (**cAP-0002GFP**) cells were selected from hBMEnds (**cAP-0002**) resistant to puromycin after transfected with GFP expressing lentivirial particles. The cells are shipped in proliferating culture with >90 confluence (the cells are provided @ passage 4-5). EGM™-2 MV full medium (contains 5% serum and growth supplements, LONZA, CC-3202) is recommended for cell culture and these cells have an average of at least additional population doubling levels >**15** when cultured following the detailed protocol described below).



Representative images of GFP-hBMEnds (Left panel: phase contrast image; Right panel: GFP image)

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**
GPF-hGEnds are negative for HIV-1, HBV, HCV, and mycoplasma.

Product Use: GFP-hBMEnds are for research use only.

Shipping: Proliferating culture in T25 flask.

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

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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 EGM™-2 MV full medium. Let the cells grow for 24 hour before subculture.

1. Subculture Protocol:

A) Coating 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 Quick Coating Solution by aspiration and the flask is ready to be used (no need for overnight incubation when coated with Quick Coating Solution).

B) Rinse the cells in T25 flask with 5ml DPBS (**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 EGM™-2 MV full 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: Don't spin the cells during the subculture process).

2. Cell culture protocol (proliferating):

A) Culture medium (EGM™-2 MV full medium) is changed every 2 days.

B) The cells normally become confluent within7 days (when split at a 1:4 ratio).

3. Preparation of quiescent cells:

A) EGM™-2MV medium containing 0.5% FBS is used to induce quiescent endothelial cells (after 18-24hours).

Caution: Handling human derived products is potentially bioharzadous. 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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