11 Park Drive, Suite 12
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GFP Expressing Human Brain Microvascular Pericytes

ORDER INFORMATION

Name of Cells: GFP Expressing Human Brain Microvascular Pericytes **(GFP-HBMVPCs)**
Catalogue Number: **cAP-0030GFP**
Product Format: Proliferating culture or Frozen cells (for international shipment)
Cell Number: > 90% confluent in T25 flask or > 5 x 10^5 cells in a frozen vial

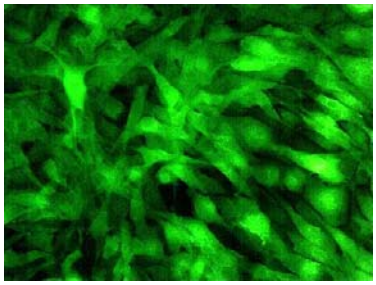
General Information

GFP-HBMVPCs are selected from GFP-expressing lentiviral particle-transfected HBMVPCs (**cAP-0030**), which are resistant to puromycin. The cells are shipped in proliferating culture with >90 confluence (the cells are provided @ passage5) or in a cryovial. Pericyte Growth Medium (cAP-09, or cAP-09B, containing FBS and growth factor supplements) is recommended for cell culture and these cells have an average population doubling capacity >10 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

GFP-HBMVPCs are negative for HIV-1, HBV, HCV, and mycoplasma.



GFP Expressing Human Brain MV Pericytes

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

Shipping status: Proliferating cells in T25 flask or in a cyrovial.

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 Pericyte Growth medium (cAP-09,

containing FBS and growth factor supplements). Let the cells grow for 24 hours before subculture.

1. Subculture Protocol:

A) Coating T25 flasks: Add 2ml 0.1% 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 PBS (**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 Trypsin/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 Pericyte Growth Medium and the cell suspension is transferred directly into 2 x pre-coated T25 flasks (10ml each, and the cells are subcultured at 1:2 ratio)

(Note: No need spin the cells during the subculture process).

2. Cell culture protocol (proliferating):

A) Culture medium: Pericyte Growth Medium should be changed every 2 days.

B) The cells normally become confluent within 7 days (when split at 1:2).

Other products needed:

Items	Company	Cat #
Quick Coating Solution	Angio-Proteomie	cAP-01
Pericyte Growth medium	Angio-Proteomie	cAP-09
PBS	Invitrogen	10010
Trypsin/EDTA	Invitrogen	25300-062

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