11 Park Drive, Suite 12
Boston, MA 02215

GFP Expressing Human Dermal Lymphatic Microvascular Endothelial Cells

ORDER INFORMATION

Name of cells: GFP Expressing Human Dermal Lymphatic Microvascular Endothelial Cells **(GFP-HDLMECs)**
Catalogue number: **cAP-0003GFP**
Product format: Proliferating culture
Cell number: > 90% confluent in T25 flask

General Information

HDLMECs (**cAP-0003**) are isolated from normal neonatal human skin tissue and transfected with GFP-Lentiviral particles at passage one. Puromycin resistant GPF-HDLMECs (cAP-0003GFP) were selected and shipped in proliferating culture with >90 confluence (the cells are provided @ passage 3). The cells are shipped in proliferating culture with >90 confluence (the cells are provided @ passage 3). ENDO-Growth medium (contains 5% serum and growth supplements, Cat# cAP-02) is recommended for cell culture and these cells have an average additional population doubling levels >**14** when cultured following the detailed protocol described below).

Characterization of the cells

Cytoplasmic CD31: **>95% positive by immunofluorescence**
Nuclear Prxo-1: **>95% positive by immunofluorescence**

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

Product Use

GFP-HDLMECs are for research use only.

Shipping

Proliferating culture in T25 flask.

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 ENDO-Growth 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 PBS (**Room Temperature, RT**) twice.

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

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 ENDO-Growth medium and the cell suspension is transferred directly into 4 pre-coated T25 flasks (5 ml each, and the cells are subcultured at 1:4 ratio)

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

2. Cell culture protocol (proliferating):

A) Culture medium (ENDO-Growth medium) is changed every 2 days.
B) The cells normally become confluent within7 days (when split at 1:4 ratio).

3. Preparation of quiescent cells:

A) ENDO-Basal medium (Cat# cAP-03) containing 0.5% FBS is used to induce quiescent endothelial cells (after 18-24hours).

Other useful information

Items	Company	Cat #
Quick Coating Solution	Angio-Proteomie	cAP-01
ENDO-Growth medium	Angio-Proteomie	cAP-02
ENDO-Basal medium	Angio-Proteomie	cAP-03
ENDO-Growth Supplement	Angio-Proteomie	cAP-04
PBS	Invitrogen	10010
Trypsin/EDTA	Invitrogen	25300-062