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Human VEGFR2 shRNA Transfected GFP-HUVECs

ORDER INFORMATION

Name of Cells:	Human VEGFR2 shRNA transfected GFP-HUVECs **(GFP-HUVECs-shVEGFR2)**
Catalogue Number:	**sAP-0001-VEGFR2-GFP**
Product Format:	Proliferating culture
Cell Number:	> 90% confluent in T25 flask

General Information

HUVECs (**cAP-0001**) were initially isolated from normal human umbilical vein and transfected with human VEGFR2-shRNA and GFP Lentiviral particles at passage one. Puromycin and Zeocin resistant **GFP-HUVECs-shVEGFR2** (**sAP-0001-VEGFR2**) were selected and shipped in proliferating culture with >90 confluence (the cells are provided @ passage 4). Endo-Growth Medium (contains 5% serum and growth supplements, cAP-02) is recommended for cell culture and these cells have an average population doubling levels >**18** when cultured following the detailed protocol described below).

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**
GFP	**>99% positive by immunofluorescence**

GFP-HUVECs-shVEGFR2 cells are negative for HIV-1, HBV, HCV, and mycoplasma.
Product Use: GFP-HUVECs-shVEGFR2 cells are for research use only.

Shipping: Proliferating culture in T25 flask.

Handling of Arriving Cells

When you receive the cells, leave the flask in a 37°C CO2 incubator for 1 hour first, and then replace the transport medium with fresh Endo-Growth Medium. Let the cells grow for 2-3 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 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 Endo-Growth 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: Never spin the cells during the subculture process).

2. Cell culture protocol (proliferating):

A) Culture medium (Endo-Growth medium) is changed every 2-3 days.

B) The cells normally become confluent within 7 days (when split with a ratio of 1:4).

3. Preparation of quiescent cells:

A) Endo-Basal Medium (cAP-03) containing 0.5% FBS is used to induce quiescent endothelial cells (after 18-24hours).