

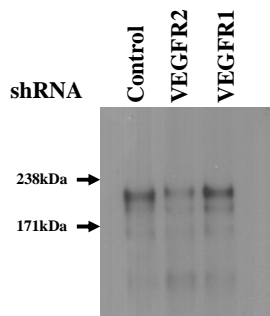
## Human VEGFR2 shRNA Transfected HUVECs

### ORDER INFORMATION

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

### General Information

HUVECs (cAP-0001) were initially isolated from normal human umbilical vein and transfected with human VEGFR1-shRNA Lentiviral particles at passage one. Puromycin resistant HUVECs-shVEGFR2 (sAP-0001-VEGFR2) were selected and shipped in proliferating culture with >90 confluence (the cells are provided @ passage 3). 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 population doubling levels >18 when cultured following the detailed protocol described below).



The levels of VEGFR2 in HUVECs-shControl, HUVECs-shVEGFR2 and HUVECs-shVEGFR1 were compared by IP (using anti human VEGFR2)-Western blotting (using anti human VEGFR2).

### 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

**HUVECs-shVEGFR2 cells** are negative for HIV-1, HBV, HCV, and mycoplasma.

**Product Use:** HUVECs-shVEGFR2 cells are for research use only.

**Shipping:** Proliferating culture in T25 flask.



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### Handling of Arriving Cells

When you receive the cells, leave the flask in 37°C CO<sub>2</sub> incubator for 1 hour first, and then replace the transport medium with fresh EGM<sup>TM</sup>-2 MV full medium. Let the cells grow for 24 hour 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 EGM<sup>TM</sup>-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: Never spin the cells during the subculture process).**

#### 2. Cell culture protocol (proliferating):

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

**Caution:** Handling human derived products is potentially biohazardous. 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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