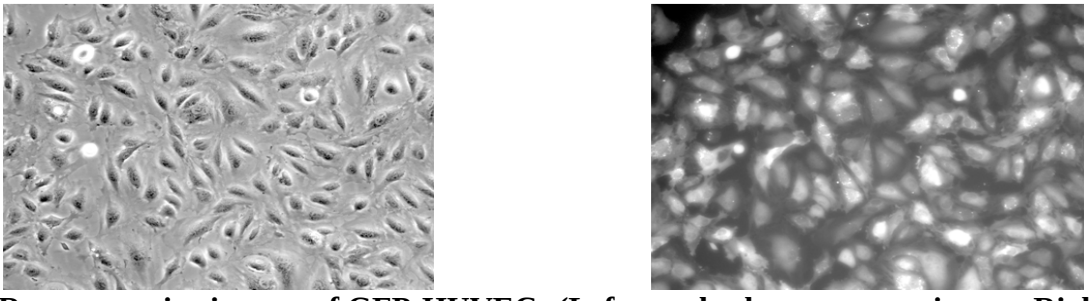
GFP Expressing Human Umbilical Vein Endothelial Cells (GFP-HUVECs)

Name of Cells:	GFP Expressing Human Umbilical Vein Endothelial Cells **(GFPhUVECs)**
Catalog No:	**GFP**
Product Format:	Proliferating culture
Cell Number:	*> 90% confluent (>5x105 cells) in T25 flask*

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.

General Information

HUVECs were isolated from normal human umbilical vein and transfected with GFP-Lentiviral particles at passage one. Puromycin resistant GPF-HUVECs (GFP) were selected and shipped in proliferating culture with >90 confluence (the cells are provided @ passage 3). ENDO-Growth medium (contains 5% serum and growth supplements, is recommended for cell culture and these cells have an average population doubling levels >**18** when cultured following the detailed protocol described below).



Representative images of GFP-HUVECs (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**

GFP-HUVECs are negative for HIV-1, HBV, HCV, and mycoplasma.
Product Use: GFP-HUVECs 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 CollaGel Hydrogel solution (**CGH320**) into one T25 flask and make sure whole surface of the flask is covered with the solution.
Five minutes later, the flask is ready to be used (no need for overnight incubation when coated with our CollaGel Hydrogel 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 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: No need 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 containing 0.5% FBS is used to induce quiescent endothelial cells (after 18-24hours).