

**GFP Expressing Human Brain Microvascular Endothelial Cells**

Catalog Number	GF2
Product Name	GFP Expressing human brain microvascular endothelial cells (GFP- HBMECs)
Storage	37°C CO2 incubator
Product Format	Proliferating culture
Cells Number	>90% confluent in T25 flask

**\*Caution:** The handling of human derived products has the potential to be biologically hazardous. All Cell strains tested negative for HIV, HBV, and HCV DNA in diagnostic tests. Proper precautions must be taken to avoid exposure. Always wear proper protective equipment (Gloves, safety glasses, etc.) when handling these materials. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination.

**GENERAL INFORMATION**

GFP expressing human brain microvascular endothelial cells are isolated from normal human brain tissue and transfected with GFP- lentiviral particles. Puromycin resistant GFP-HBMECs were selected and shipped in proliferating culture or frozen vial with a confluence of > 90% (cells are providing at passage 4-5). ENDO-Growth medium (EGM-2102) containing 5% serum and growth supplement is recommended for culture. Cells have an average population doubling level >15 when cultured. When you receive the cells, leave the flask in 37°C CO2 incubator for 1 hour. Then, replace the transport medium with fresh ENDO-Growth medium (EGM-2102). Let the cells grow for 24 hours before subculture.

**CELL CHARACTERIZATION**

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 Expressing human brain microvascular endothelial cells are negative for	HIV-1, HBV, HCV, and mycoplasma

**PRODUCT USE AND SHIPPING STATUS**

Product Use	GFP Expressing human brain microvascular endothelial cells are for research use only
Shipping Status	Proliferating culture in T25 flask

**T25 flask**

\*Coating T25 flasks. Add 2 ml AlphaBioCoat (AC001) into 3- T25 flasks and ensure entire interior surface is coated with the solution. After 30 minutes, dispose of AlphaBioCoat (AC001) by aspiration. Gently rinse and aspirate the flask with Phosphate Buffer Solution (1XPBS-001). The flask is now ready for use (no need for overnight incubation when coated with AC001). Add fresh media to flask, if color changes from pink to yellow, discard the media, and add fresh media to each flask.

1. Inspect to make sure Flask is at 90% confluence, if not remove transport media, and add 5ml of fresh media to the flask. Place flask in 37°C incubator until cells are at 90% confluence. Change media every 2 days.
2. If flask is at 90% confluence, aspirate transport media from flask.
3. Rinse T25 flask containing cells with 5 ml 1XPBS.
4. Gently aspirate out the PBS after rinsing, and discard.
5. Add 2ml of RT trypsin/ EDTA to T25 flask containing cells (ensure entire interior surface is covered).
6. Place T25 flask containing cells into 37°C incubator for 1 or 2 minutes (cells will normally come off of the surface within 1 or 2 minutes).
7. Suspend the cells with 15ml of ENDO-Growth medium (EGM-2102) and transfer equally into 3 pre-coated T25 flasks (the cells are now at a subculture ratio of 1:3).
8. There is no need to spin cells during subculture.
9. Proliferating cells culture: ENDO-Growth medium (EGM-2102) should be changed every 2 days. The cells normally become confluent within 7 days (when split at a 1:3 ratio).
10. Use ENDO- Basal media (EBM-002) containing 0.5% FBS to induce quiescent cells (after 18-24 hours).