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**RFP Expressing Human Brain Microvascular Endothelial Cells**

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Catalog Number	RFP2
Product Name	RFP Human Brain Microvascular Endothelial Cells (RFP-HBMVPCs)
Storage	Liquid Nitrogen
Product Format	Frozen vial
Cells Number	>90% confluent in Frozen vial

**\*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**

RFP expressing human Brain Microvascular Endothelial Cells were isolated from normal human brain tissue and transfected with RFP-Expressing lentiviral particles at passage 1. Puromycin resistant, RFP-HBMVPCs is shipped in proliferating culture or frozen vial with a confluence of > 90% (cells are provide @ passage 4-5). Endo-Growth medium (EGM2102) containing 5% serum and growth supplement is recommended for culture. Cells have an average population doubling level >10 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 (EGM2102). Let the cells grow for 24 hours before subculture.

**CELL CHARACTERIZATION**

Cytoplasmic Alpha-Actinin Filaments	>80% positive by immunofluorescence
Cytoplasmic Desmin Intermediate Filaments	>80% positive by immunofluorescence
Cytoplasmic Uptake of Di-I-Ac-LDL	<2% Positive by immunofluorescence
Cytoplasmic VWF/ Factor VIII	<2% positive by immunofluorescence
RFP expressing human brain microvascular endothelial cells are negative for	HIV-1, HBV, HCV, and mycoplasma

**PRODUCT USE AND SHIPPING STATUS**

Product Use	RFP expressing human brain microvascular endothelial cells are for research use only
Shipping Status	Frozen vial

**Frozen**

- 1) Coating T25 flasks. Add 2 ml AlphaBioCoat (AC001) into a T25 flask and ensure entire interior surface is coated with the solution. After 30 minutes, dispose of AlphaBioCoat (AC001) by aspiration. Gently rinse and aspirate flask with Phosphate Buffer Solution (1XPBS-001). The flask is now ready for use (no need for overnight incubation when coated with AC001).
- 2) If you are using the coated flask the same day, add about 4 ml of ENDO-Growth media (EGM2102) to the coated flask. If the media changes color from pink to yellow, aspirate and discard the media. Add 4ml of fresh media to the coated flask.
- 3) Thaw the cells in a 37°C water bath. Once you see a small amount of ice left in the vial, spray the vial with 70% Ethanol and wipe it down.
- 4) Transfer the vial into your Biosafety cabinet.
- 5) Using a 2 or 5ml pipet, pipet the cells out of the vial.
- 6) Transfer your cell suspension in to your coated plate that have the 4 ml media in it.
- 7) You should have a total working volume of 5ml of cell suspension in the flask; close the cap. Make sure cells are evenly distributed in the flask by moving the flask left and right five times. Move it up and down for and additional five times.
- 8) Place flask in a 37°C incubator with 5% CO<sub>2</sub>. If flask is not vented, please loosen cap.
- 9) Change media after 48 hours.