NEUROMICS

Human Brain Microvascular Endothelial Cells Expressing VE-CAD-GFP

Catalog #: HEC02-VE-CAD-GFP

Cell #: > $5X10^5$ cells

Storage: Liquid Nitrogen until ready for culture.
While culturing keep in 37°C CO_2 Incubator

Product Format: Frozen

GENERAL INFORMATION

HBMVECs cells were isolated from the normal human cerebral cortex. HBMVEC-VE-CAD-GFP were selected by puromycin from HBMVECs after infected with lentiviruses expressing VE-CAD-GFP the cells are shipped in frozen vials (the cells are provided @ passage 1). Endo-Growth Media (MED001, contains 10% serum and growth supplements) is recommended for cell culture and these cells have a minimum average population doubling levels > 15 when cultured following the detailed protocol described below).

Product is for research use only.

Frozen vials are shipped in a dry ice package.

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

HBMVECs are negative for HIV-1, HBV, HCV, and mycoplasma.

HANDLING OF ARRIVING CELLS

When you receive the cells in a frozen vial, you can transfer the vial of cells into a -80C freezer for short period storage or a liquid nitrogen tank for long-term storage. Thaw the cells in a 37C water bath, and then transfer the cells into a T25 flask pre-coated with Smooth Coat Solution (SC300) as described in following details.

PROTOCOL FOR THAWING THE CELLS AND SUBCULTURE

Note: If you have any questions or need clarification regarding the protocol for culturing these cells, please reach out to Dr. Jensen Auguste at (978) 608-1766 with your questions before beginning.

1. Pre-coating of T25 flasks - Add 2ml each Smooth Coat Solution (SC300) into a T25 flask to cover the whole surface of the flask, 5 mins later, dispose the excessive coating solution by aspiration and the flask is ready to be used (although solution containing other extracellular matrix, i.e. gelatin, collagen, and fibronectin, can be used, make sure to optimize the conditions in advance).

2. Thaw the frozen cell vial in a 37C water bath first, and then transfer the cells into the pre-coated T25 flask with 10ml of medium, cells usually become confluent overnight and ready to be passaged.

3. To passage the cells, rinse the cells in a T25 flask with 5ml HBSS (RT) twice; then add 2ml Universal Detachment Solution (AD002) into one T25 flask; gently dispose the excessive solution by aspiration.

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4. Leave the T25 flask with the cells at RT or 37C for about 1 min (most cells usually will detach from the surface within 1-2 mins; or monitor the cells under a microscope until most of cells become rounded up, and then gently tap the flask against the bench surface, and the cells will move on the surface of the flask when monitoring under microscope.

5. Add 5ml Universal Neutralization Buffer (NB001) and spin down the cells with 800g centrifugation for 5 mins.

6. Re-suspend the cell pellet with 10 or 15 ml medium and transfer 5 ml each into 2 or 3 pre-coated T25 flasks (for 1/2 to 1/3 subculture ratio).

7. Change medium every 2 or 3 days and the cells usually become confluent within 7 days (when split at a 1/3 Ratio).

8. To prepare quiescent cells, when cells are nearly confluent, replace Endo-Growth Media (MED001) with Endo-Basal Media (MED002) containing 0.5%FBS for about 8-12hrs before your experiments.

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