NEUROMICS

ACE2-GFP Human Pulmonary Artery Endothelial Cells

Catalog #: HEC24

Cell #: >5x10^5 cells

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

Product Format: Frozen Vial

GENERAL INFORMATION

HPuAECs cells were isolated from normal human pulmonary Artery. ACE2-GFP Expressing Human Pulmonary Artery Endothelial Cells were selected by puromycin resistant HPuAEcs after infected with lentiviruses expressing ACE2-GFP. The cells are shipped in frozen vials (the cells are provided @ passage 1). Endo-Growth Medium (MED001), contains 10% serum and growth supplements, is recommended for cell culture and these cells have a minimum average population doubling levels > 16 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

1. Cytoplasmic VWF/Factor VIII >95% positive by immunofluorescence

2. Cytoplasmic uptake of Di-I-Ac-LDL >95% positive by immunofluorescence

3. Cytoplasmic PECAM1 >95% positive by immunofluorescence

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

HANDLING OF ARRIVING CELLS

When you receive the dry ice package with cells in frozen vials, transfer the frozen vials of cells into a -80ºC freezer for short period storage or a liquid nitrogen tank for long-term storage.

PROTOCOL FOR THAWING THE CELLS AND SUBCULTURE

1. Pre-coating of T25 flasks- Add 2ml each Alphabiocoat Solution (AC001) into a T25 flask to cover the whole surface of the flask, 30 mins later, remove excessive coating solution by aspiration. Rinse with 1xPBS and flask is ready to be used.

2. Thaw the frozen cell vial in a 37ºC water bath first, and then transfer the cells into the pre-coated T25 flask with 10ml of Endo-Growth Medium (MED001), cells usually become confluent with 1-2 days and ready to be passaged.

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3. To passage the cells, rinse the cells in a T25 flask with 5 ml HBSS (RT) twice; then add 2 ml Universal Detachment Solution (RT) (AD002) into one T25 flask; gently dispose the excessive Universal Detachment Solution within 20 seconds by aspiration.

4. Leave the T25 flask with the cells at RT or 37ºC for 1 min. (Most cells usually will detach from the surface within 1-2 mins.) Otherwise, 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.

5. Add 5 ml 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 with Endo Basal Medium (MED002) containing 0.5%FBS for about 8-12hrs

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