



## ACE2-GFP Human Lung Airway Smooth Muscle Cells

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**Catalog #:** HMC001

**Cell #:** >5x10<sup>5</sup> cells

**Storage:** Liquid Nitrogen until ready for culture.  
While Culturing keep in 37°C CO<sub>2</sub> incubator

**Product Format:** Frozen Vial

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### GENERAL INFORMATION

HLAWSMCs are isolated from human normal lung small airway tissues. Human Lung Airway Smooth Muscle Cells Expressing ACE2-GFP (HLAWSMCs-ACE2-GFP) were selected from puromycin resistant HLAWSMCs cells after infected with lentiviruses expressing ACE2-GFP and the cells are shipped in frozen vials (provided @ passage 1). Smooth Muscle Growth Medium (contains FBS and Growth factor supplements, SMCM002) is recommended for cell culture and these cells have a minimum average population doubling levels > 8 (2-3 passages) 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. Alpha-Vascular SMA > 98% positive by immunofluorescence
2. VE-Cadherin < 1% positive by immunofluorescence
3. HLAWSMCs 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 -80°C 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 2 ml each Alphabiocoat (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 37°C water bath first, and then transfer the cells into the pre-coated T25 flask with 10ml of Smooth Muscle Growth Medium (SMCM002), 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 37°C 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 Smooth Muscle Basal Medium (SMCM001) containing 0.5%FBS for about 8-12hrs.

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