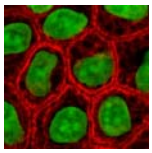

AddexBio
Research Services

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Specification Sheet: Human Idiopathic Pulmonary Arterial Hypertension Smooth Muscle Cells (HIPAHSMC)

Catalog #: P0016002

Specification:
Idiopathic pulmonary arterial hypertension (IPAH) is the term for pulmonary arterial hypertension of unknown etiology and it represents pulmonary vascular disease with a spectrum of clinical presentations. Pulmonary vascular remodeling due to overgrowth of pulmonary artery smooth muscle cells (PASMC) is a major cause for the elevated vascular resistance in patients with idiopathic pulmonary arterial hypertension (IPAH). Smooth muscle cells (SMC) are important component of arterial disease. The increased growth potential of vascular SMC represents one of the crucial anomalies responsible for the development of essential vascular diseases. New studies demonstrate that SMC express calcium channels . The HIPAHSMCs from Addexbio are isolated from the human pulmonary artery of IPAH patients. These cells are cryopreserved at secondary culture after purification and delivered frozen. Each vial contains > 1x10^6 cells in 1 ml volume. HIPAHSMC are characterized by immunofluorescent method with antibodies to smooth muscle α-actin and desmin. HIPAHSMC are tested negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast, and fungi. Growth performance is tested through multiple passages up to 15 population doublings (PD) under culture conditions without antibiotics.

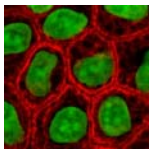
Recommended Medium:
SMC Medium (AddexBio, Cat #: M00125) supplemented with SMCGS (AddexBio, Cat #: M00125a)

Intended Use:
HIPAHSMC are for research use only. It is not approved for human or animal use, or for application in *in vitro* diagnostic or therapeutic procedures.

Precaution:
Handling human derived products is potentially bioharzadous. Although each cell strain testes negative for HIV, HBV and HCV DNA, mycoplasma, bacteria, fungi and yeast, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions must be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination.

Storage:
Directly and immediately transfer cells from dry ice to liquid nitrogen upon receiving and keep the cells in liquid nitrogen until cell culture needed for experiments.

Shipping:
The vial is shipped on dry ice.



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Culture Initiation from Cryopreserved Cells:

1. Prepare a poly-lysine coated flask. Add 5 ml of sterile cell culture-grade poly-lysine stock solution (AddexBio, Cat #: M001331). Leave the flask in incubator or biosafety cabinet for 0.5 hour.

2. Prepare complete medium: decontaminate the external surfaces of the medium and medium supplements with 70% ethanol and transfer them to sterile field. Aseptically open each supplement tube and add them to the basal medium with a pipette. Rinse each tube with medium to recover the entire volume.

3. Aspirate poly-lysine solution and rinse the flask with sterile cell culture-grade water twice. Add 20 ml of complete medium to the flask. Leave the flask in the hood and go to thaw the cells.

4. Place the vial in a 37$_{o}$C waterbath, hold and rotate the vial gently until the contents are completely thawed. Remove the vial from the waterbath immediately, wipe it dry, rinse the vial with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads with fingers. Using 1 ml eppendorf pipette gently resuspend the contents of the vial.

5. Dispense the contents of the vial into the equilibrated, poly-lysine coated culture vessels. A subculture ratio of 1:3 to 1:5 is recommended. (Dilution and centrifugation of cells after thawing are not recommended)

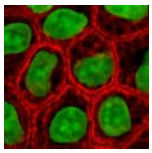
6. Replace the cap or cover, and gently rock the vessel to distribute the cells evenly. Return the culture vessels to the incubator.

7. For best result, do not disturb the culture for at least 16 hours after the culture has been initiated. Change the growth medium the next day to remove the residual DMSO and unattached cells, then every other day thereafter. A healthy culture will display spindle shaped, usually in a homogeneous bundle or sheet of cells rather than scattered single cells and the cell number will be doubled after two to three days in culture.

Culture Maintenance:

1. Change the medium to fresh supplemented medium the next morning after establishing a culture from cryopreserved cells. For subsequent subcultures, change medium 48 hours after establishing the subculture.

2. it is recommended to change the medium every day or at least every other day.



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Subculture:

1. Subculture the cells when they reach 80-90% confluent.

2. Prepare poly-lysine coated cell culture flasks.

3. Warm medium, trypsin/EDTA solution (0.05%), and DPBS to room temperature.

4. Rinse the cells with DPBS.

5. Incubate cells with 1-2 ml of trypsin/EDTA solution (depending on the size of the flask) until most of the cells are rounded and detached.

6. Rinse the flask with 10 ml complete growth medium to harvest the cells and transfer to a 15-ml centrifuge tube.

7. Centrifuge the cell suspension at 1000 rpm for 5 min at room temperature and resuspend the cells in complete growth medium.

8. Plate the cells in a new and poly-lysine coated flask at 1:3 to 1:5 ratio.