Human Umbilical Vein Smooth Muscle Cells (HUVSMC) provided by Innoprot are isolated by ScienCell Research Laboratories from human umbilical vein. HUVSMC are cryopreserved at the end of secondary culture and delivered frozen and are guaranteed to further expand for 15 population doublings in the condition provided by ScienCell Research Laboratories.

Vascular smooth muscle cell is the cellular substate of most significant arterial disease. The increased growth potential of vascular smooth muscle cells represents one of the crucial anomalies responsible for the development of essential vascular diseases. New studies demonstrate that vascular smooth muscle cells express ICAM-1 and VCAM-1, which may contribute to the inflammatory reaction in the vascular wall and may actively be involved in the progression and stability of vascular disease. In vitro culture of human VSMC as a model of vascular research played a critical role and continues providing information in the pharmacology and the therapy of vascular diseases.

**Recommended Medium**
- Smooth Muscle Cell Medium (Reference: P60125)

**Product Characterization**
Immunofluorescent method
- \( \alpha \)-smooth muscle actin
- Desmin

The cells test negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi

**Product Use**
THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in vitro diagnostic or clinical procedures.
INSTRUCTIONS FOR CULTURING CELLS

**IMPORTANT:** Cryopreserved cells are very delicate. Thaw the vial in a 37 ºC waterbath and return them to culture as quickly as possible with minimal handling!

**Set up culture after receiving the order:**

1. Place the vial in a 37ºC waterbath, hold and rotate the vial gently until the contents are completely thawed. Remove the vial from the waterbath immediately, wipe it dry, and transfer it to a sterile field. Rinse the vial with 70% ethanol, and then wipe to remove excess. 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.

2. Dispense the contents of the vial into the equilibrated, poly-L-lysine coated culture vessels. A seeding density of 5,000 cells/cm² is recommended. Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of DMSO residue in the culture. It is also important that vascular smooth muscle cells are plated in poly-L-lysine coated culture vessels that promote vascular smooth muscle cell attachment.

3. Replace the cap or cover, and gently rock the vessel to distribute the cells evenly. Loosen caps if necessary to permit gas exchange.

4. Return the culture vessels to the incubator.

5. 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 health 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.

**Maintenance of Culture:**

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. Change the medium every other day thereafter, until the culture is approximately 50% confluent.

3. Once the culture reaches 50% confluence, change medium every day until the culture is approximately 80% confluent.

**Subculture:**

1. Subculture the cells when they are 80% confluent.

2. Prepare poly-L-lysine coated cell culture flasks.

3. Warm medium, trypsin/EDTA solution, trypsin neutralization solution, and DPBS to room temperature. We do not recommend warming the reagents and medium at 37ºC waterbath prior to use.

4. Rinse the cells with DPBS.

5. Incubate cells with 3 ml of trypsin/EDTA solution (in the case of T-25 flask) until 80% of cells are rounded up (monitored with microscope). Add 3 ml of trypsin neutralization solution to the digestion immediately and gently rock the culture vessel.
6. Harvest and transfer released cells into a 15 ml centrifuge tube. Rinse the flask with another 3 ml of growth medium to collect the residue cells. Examine the flask under microscope to make sure the harvesting is successful by looking at the number of cells left behind. There should be less than 5%.

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

8. Count cells and plate them in a new, poly-L-lysine coated flask with cell density as recommended.

**Caution**: Handling human derived products is potentially biohazardous. Although each cell strain tests negative for HIV, HBV and HCV DNA, 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 [1].