Human Esophageal Smooth Muscle Cells (HESMC)

Catalog Number: 2710

Cell Specification

Smooth muscle is responsible for the contractility of hollow organs, such as blood vessels, the gastrointestinal tract, the bladder, and the uterus. Its structure differs greatly from that of skeletal muscle. The human esophagus contains three layers of muscle in its walls, the outer longitudinal and inner circular layers of the main muscular coat and the muscular layer of the mucosa. Visceral smooth muscle cells makes up all three layers along the entire organ except for the most rostral few centimeters. Smooth muscle contraction is critical to peristalsis in the human esophagus and the contraction may be mediated by activation of phospholipase through two distinct mechanisms (increased intracellular Ca2+ and G protein activation) and activating PKCepsilon-dependent mechanisms [1]. *In vitro* study also shows that cultured human esophageal smooth muscle cells express voltage-dependent Na(+) channels [2].

HESMC from ScienCell Research Laboratories are isolated from human esophagus. HESMC are cryopreserved at secondary culture and delivered frozen. Each vial contains >5 x 10^5 cells in 1 ml volume. HESMC are characterized by immunofluorescent method with antibodies to α-smooth muscle actin and desmin. HESMC are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi. HESMC are guaranteed to further expand for 15 population doublings at the condition provided by ScienCell Research Laboratories.

Recommended Medium

It is recommended to use smooth muscle cell medium (SMCM, Cat. No. 1101) for the culturing of HESMC *in vitro*.

Product Use

HESMC are for research use only. It is not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

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

Dry ice.

Reference

[1] Cao, W., Chen, Q., Sohn, U. D., Kim, N., Kirber, M. T., Harnett, K. M., Behar, J., Biancani, P. (2001) Ca2+-induced contraction of cat esophageal circular smooth muscle cells. *Am J Physiol Cell Physiol*. 280(4):C980-92.

[2] Deshpande, M. A., Wang, J., Preiksaitis, H. G., Laurier, L. G., Sims, S. M. (2002) Characterization of a voltage-dependent Na(+) current in human esophageal smooth muscle. *Am J Physiol Cell Physiol*. 283(4):C1045-55.

Instruction for culturing cells

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

Set up culture after receiving the ordering:

1. Place the vial in a 37°C 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 7,500 cells/cm^2 is recommended.
3. *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 HESMC are plated in poly-L-lysine coated culture vessels that promote cell attachment and growth.*
4. Replace the cap or cover, and gently rock the vessel to distribute the cells evenly. Loosen caps if necessary to permit gas exchange.
5. Return the culture vessels to the incubator.
6. 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 37°C 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. *Note: Use ScienCell Research Laboratories' trypsin/EDTA solution that is optimized to minimize the killing of the cells by over trypsinization.*
7. 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%.
8. Centrifuge the harvested cell suspension at 1000 rpm for 5 min and resuspend cells in growth medium.
9. 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 bioharzadous. Although each cell strain testes negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions mush 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].

[1]. Grizzle, W. E., and Polt, S. S. (1988) Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues. J Tissue Culture Methods. 11(4).