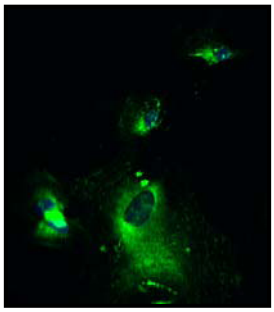
株式会社ケーエーシー

KAC

REF: P10406

CARDIAC CELL SYSTEM INNOPROFILE™

RAT AORTIC SMOOTH MUSCLE CELLS



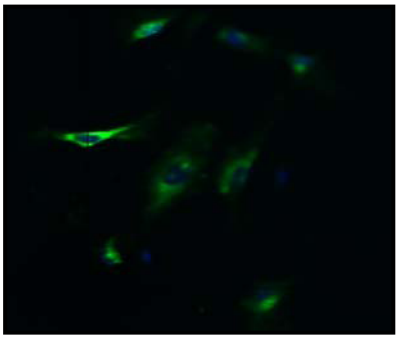
Product Type:	Cryo-preserved Smooth Muscle Cells
Catalog Number:	P10406
Source:	Rat Aorta (Sprague Dawley)
Number of Cells:	5 x 10^5 Cells / vial (1ml)
Storage:	Liquid Nitrogen

Rat Aortic Smooth Muscle Cells (RASMC) are isolated from adult Sprague Dawley rat aorta. RASMC are cryopreserved from passage one culture. RASMC are guaranteed to further expand for 15 population doublings at the conditions recommended in the instructions provided by Innoprot.

Smooth muscle cell (SMC) is the cellular substrate of most significant 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 and the expression of ICAM-1 and VCAM-1 on SMC 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 vascular SMC as a model of vascular research played a critical role and continues providing information in the pharmacology, drug discovery and the therapy of vascular diseases.

Recommended Medium

- RPMI1640 + FBS(10%)



Product Characterization

Immunofluorescent method

- α -smooth muscle actin

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

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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. 1. Place the vial in a 37 ºC 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, collagen coated culture vessels. A seeding density of 10,000 cells/cm^2 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 collagen coated culture vessels that promote vascular smooth muscle cell attachment and growth.*

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 over 90% 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 ºC waterbath prior to use.

4. Rinse the cells with DPBS.

5. Incubate cells with 10 ml of trypsin/EDTA solution (in the case of T-75 flask) until 80% of cells are rounded up (monitored with microscope). Add 10 ml of trypsin neutralization solution to the digestion immediately and gently rock the culture vessel.

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6. Harvest and transfer released cells into a 50 ml centrifuge tube. Rinse the flask with another 10 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, collagen 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).

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