ScienCell™
Research Laboratories

Smooth Muscle Cell Medium-serum free (SMCM-sf)

Catalog Number: 1111

Product Description

Smooth muscle cell-serum free medium (SMCM-sf) is designed for optimal growth and expansion of normal human smooth muscle cells (SMCs) *in vitro*. It is a sterile, liquid medium which contains essential and non-essential amino acids, vitamins, organic and inorganic compounds, hormones, growth factors, trace minerals. The medium is HEPES and bicarbonate buffered and has a pH of 7.4 when equilibrated in an incubator with an atmosphere of 5% CO_2/95% air. The medium is formulated (quantitatively and qualitatively) to provide a defined and optimally balanced nutritional environment at serum-free condition that selectively promotes growth of normal human smooth muscle cells *in vitro*.

Components

SMCM-sf consists of 500 ml of basal medium, 5 ml of smooth muscle cell growth supplement serum free (SMCGS-sf, Cat. No. 1162) and 5 ml of penicillin/streptomycin solution (P/S, Cat. No. 0503).

Product Use

SMCM-sf is for research use only. It is not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Storage

Store the basal medium at 4ºC, the SMCGS-sf and the P/S solution at -20ºC. Protect from light.

Shipping

Gel ice.

Prepare for use

Thaw SMCGS-sf and P/S solution at 37ºC. Gently tilt the SMCGS-sf tube several times during thawing to help the contents dissolve. **Make sure the contents of the supplement are completely dissolved into solution before adding to the medium**. Rinse the bottle and tubes with 70% ethanol, and then wipe to remove excess. Remove the cap, being careful not to touch the interior threads with fingers. Add SMCGS-sf and P/S solution into basal medium in a sterile field, mix well and then the reconstituted medium is ready for use. Since several components of SMCM-sf are light-labile, it is recommended that the medium not be exposed to light for lengthy periods of time. If the medium is warmed prior to use, do not exceed 37ºC. When stored in the dark at 4ºC, the reconstituted medium is stable for one month.

Caution: If handled improperly, some components of the medium may present a health hazard. Take appropriate precautions when handling it, including the wearing of protective clothing and eyewear. Dispose of properly.

Instruction for culturing cells in SMCM-sf

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 order:

1. Prepare a poly-L-lysine coated flask (2 μg/cm^2, T-75 flask is recommended) and leave the flask in incubator overnight (minimum one hour at 37°C incubator).

2. Prepare smooth muscle cell medium-serum free (SMCM-sf Cat. No. 1111): decontaminate the external surfaces of 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. Rinse the poly-L-lysine coated flask with sterile water twice and 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°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-L-lysine 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 cells are plated in poly-L-lysine coated culture vessels that promote smooth muscle cell attachment.

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

7. Return the culture vessels to the incubator.

8. For best result, do not disturb the culture for at least 16 hours after the culture has been initiated. Change the medium the next day to remove the residual DMSO and unattached cells, then every other day thereafter. 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 cells SMCM-sf:

1. Subculture the cells when they are 90% confluent (It should be about 4-5 days after last passage, if a seeding density of 10,000 cells/cm^2 is used).

   Note: Over-confluent culture may cause cell death. In addition, initiating subculture under this condition may affect medium performance.

2. Prepare poly-L-lysine coated cell culture flasks (2 μg/cm^2).
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 9 ml of DPBS and 1 ml of trypsin/EDTA solution (in the case of T-75 flask) until 80% of cells are rounded up (monitored with microscope. DO NOT over-trypsinize the cells). Add 10 ml of trypsin neutralization solution to the digestion immediately and gently tap the culture vessel.
   Note: Use ScienCell Research Laboratories' trypsin/EDTA solution that is optimized to minimize the killing of the cells by over-trypsinization.
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 the 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 ***>=10,000 cells/cm^2*** seeding density.

   Note: The optimal cell expansion was obtained at >= 10,000 cells/cm^2. Reduced seeding density may affect cell growth in serum free medium.

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).