Human Vascular Smooth Muscle Cell Line Maintenance

Catalogue Numbers: CLU305 (HITB5), CLU306 (HITC6) and CLU307 (HITD5)

***Note: When purchasing one of the cell lines you can expect to receive a vial with a passage number on the range of 25-27. Due to this, it is critical that you run your experiments at the same time as generating seed stock.

If properly maintained, it has been reported that the cells will replicate until about the 35th subculture, at which time they will senesce - i.e. they will remain alive but they will no longer replicate. They will gradually slow their replication rate and be less dense at confluence, before that time. However, be advised that we cannot guarantee sufficient cell density and replication of the cells up to the 35th subculture and repurchase of the cells may be necessary.

The use of specialized growth media with supplements, such as the SmGM-2 BulletKit from Lonza, may be used to extend the cells life beyond the 35th subculture.

Description:

The HITB5, HITC6 and HITD5 clones were generated from primary cultures of human smooth muscle cells prepared from internal thoracic artery. These cells assume a proliferative, motile phenotype when cultured in M199 media in the presence of 10% FBS. When serum deprived the cells no longer proliferate but assume an elongated spindle-shaped morphology with suppressed motility. The serum deprived cells are also seen to contract in vitro in response to the vasoactive hormones histamine and angiotensin II.

These cell lines may be valuable for clarifying our understanding of SMC phenotype switching and restructuring of the vessel wall. Additionally, these cell lines are ideal for studies involving heart disease, stroke, angiogenesis and vasculogenesis, drug development, toxicity, cell-cell interactions, wound healing and cancer therapy.

Recommended media requirements:

Standard Growth Media

M199, HEPES: Life Technologies, 12340 (with Earle’s salts, L-glutamine, sodium bicarbonate (2.2 g/L) and 25 mM HEPES)
Fetal Bovine Serum, Qualified (CAN): Life Technologies, 12483-020
Alternative Fetal Bovine Serum, Qualified (US): Life Technologies, 16140-071
L-Glutamine (200 mM): Life Technologies, 25030-081
Penicillin-Streptomycin, Liquid: Life Technologies, 15140-122, contains 10,000 units of penicillin and 10,000 μg of streptomycin/ml
0.25% Trypsin-EDTA (1X), Phenol Red: Life Technologies, 25200-056

Specialized Growth Media with Supplements

SmGM-2 BulletKit (CC-3181 & CC-4149): Lonza, CC-3182
Penicillin-Streptomycin, Liquid: Life Technologies, 15140-122, contains 10,000 units of penicillin and 10,000 μg of streptomycin/ml
0.25% Trypsin-EDTA (1X), Phenol Red: Life Technologies, 25200-056
Cell culture conditions:
Complete culture medium: M199 media (cat# 12340) + 2mM L-glutamine + 100 µg penicillin and 100 µg streptomycin + 10% fetal bovine serum (FBS)*.
*It is critical that the FBS used is heat-inactivated, if not the cells will not grow well and will take much longer to reach confluence. Additionally, we strongly recommend that one of the two versions of FBS above be used in the media as only they have been validated. This cell line is quite sensitive and the use of non-validated FBS from other suppliers can adversely affect growth.

Or

Complete culture medium: SmGM-2 BulletKit (Cat# CC-3182) + 100 µg penicillin and 100 µg streptomycin

1. Thaw SMCs (1 ml total) in the cryotubes in 37°C water until thawed. Equilibrate 1 ml of cells in 1 ml of M199 medium or SmGM-2 for 1 min. then add 7 ml of fresh medium. Centrifuge at 800 rpm for 6 min. Discard supernatant and resuspend the cells in 10 ml of culture medium. Plate the cells from one cryotube onto one 100 mm tissue culture dish.

2. As the cells grow, supplement the cells with 4 mls of fresh media every other day until the cells reach 60% confluence. Do not give the cells a complete media change until this point. Once the cells are over 60% confluence, change the medium every other day until the cells reach confluence.

Avoid bringing the cells to post-confluence.

3. When the cells have reached confluence, split by washing once in sterile PBS and dissociate with 4 ml of 0.25% trypsin, 1 mM EDTA (cat# 25200-056). Watch the cells under the microscope and when they start to round up then add 6 ml of complete medium (10% FBS) to neutralize the trypsin activity. Gently dislodge cell with a pipette and put them into a sterile tube, centrifuge at 800 rpm for 6 min. and resuspend the cell pellet in 4-6 ml of culture medium. Plate cells in two or three 100 mm tissue culture dishes by adding 2 ml of cells to the center of a dish containing 8 ml of media. Once the cells are added gently rock the dishes front to back and side to side to ensure an even distribution within the plate.

Note: If cells are exposed to trypsin for too long, their morphology and performance will be affected long-term. The cells should take 5-7 days to reach confluence. If they are taking longer than this then only do a ½ split to make sure the cells are content.

Cell line freezing:

4. Cells can be frozen down in freezing medium (90% FBS (cat# 12483-020) and 10% DMSO) at 10^6 cells (a confluent 100 mm dish)/vial. Store vials at -80°C for a few days an transfer cells into liquid nitrogen for long-term storage. Cell viability decreases significantly when stored at -80°C for more than 6 months.

References:
