AC16 Human Cardiomyocyte Cell Line

Immortalized Cell Line

Cat. # SCC109

Pack size: ≥1X10^6 viable cells/vial

Store in liquid nitrogen

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.
THIS PRODUCT CONTAINS GENETICALLY MODIFIED ORGANISMS.

Certificate of Analysis

Background

AC16 is a proliferating human cardiomyocyte cell line that was derived from the fusion of primary cells from adult human ventricular heart tissues with SV40 transformed, uridine auxotroph human fibroblasts, devoid of mitochondrial DNA [1, 2]. After selection in uridine-free medium to remove unfused fibroblasts, the resulting fused cells were further subcloned and subsequently screened for the presence of SV40 large T-ag, β-myosin heavy chain (βMHC) and connexin-43 (CX-43) [1].

AC16 can be serially passaged and can differentiate when cultured in mitogen-free medium. AC-16 can be used to address questions of cardiac biology at the cellular and molecular levels.

Short Tandem Repeat (STR) Profile

D3S1358: 17, 18	D16S539: 11, 13
TH01: 7, 8, 9.3	CSF1PO: 9, 11, 12
D21S11: 32.2, 33.2	Penta D: 2.2, 9
D18S51: 12, 17	vWA: 16, 18
Penta E: 7, 8, 16	D8S1179: 12, 14
D5S818: 9, 11	TPOX: 11
D13S317: 12, 13	FGA: 21, 25
D7S820: 10, 11, 12	Amelogenin: X

Cell lines are inherently genetically unstable. Genetic instability may arise in the form of loss of heterozygosity of alleles at one or more genetic sites with increased passages.

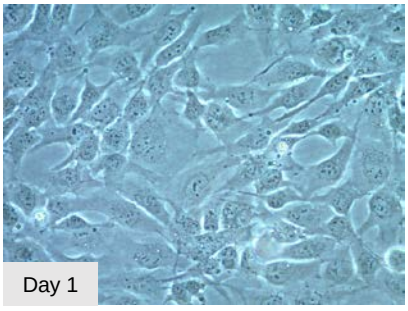
Storage and Handling

AC16 Human Cardiomyocyte Cell Line should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

Quality Control Testing

- Each vial contains ≥ $1X10^6$ viable cells.
- Cells are tested negative for HPV-16, HPV-18, Hepatitis A, B, C, and HIV-1 & 2 viruses by PCR.
- Cells are negative for mycoplasma contamination.
- Each lot of cells is genotyped by STR analysis to verify the unique identity of the cell line.

Data



References

1. Davidson, M. M., Nesti, C., Palenzuela, L., Walker, W. F., Hernandez, E., Protas, L., Hirano, M., Isaac, N. D. (2005) Novel cell lines derived from adult human ventricular cardiomyocytes. *J. Mol. Cell Cardiol.* 39(1): 133-147.

2. Litzkas, P., Jha, K.K., Ozer, H.L. (1984) Efficient transfer of cloned DNA into human diploid cells: protoplast fusion in suspension. *Mol. Cell. Biol* 4(11): 2549-2552.

SPECIES LEGEND: **H** Human **Ca** Canine **M** Mouse **R** Rat **Rb** Rabbit **B** Bovine **P** Porcine **WR** Most Common Vertebrates

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Protocols

Thawing Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells can grow on normal tissue culture ware surfaces without any additional coating.

   Cells are thawed and expanded in DMEM/F12 (Sigma Cat. No. D6434) containing 2 mM L-Glutamine (EMD Millipore Cat. No. TMS-002-C), 12.5% FBS (EMD Millipore Cat. No. ES-009-B) and 1X Penicillin-Streptomycin Solution (EMD Millipore Cat. No. TMS-AB2-C).
2. Remove the vial of frozen AC16 cells from liquid nitrogen and incubate in a 37°C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

   IMPORTANT: Do not vortex the cells.
3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In a laminar flow hood, use a 1 or 2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of AC16 Expansion Medium (Step 1 above) to the 15 mL conical tube.

   IMPORTANT: Do not add the entire volume of medium all at once to the cells. This may result in decreased cell viability due to osmotic shock.
6. Gently mix the cell suspension by slowly pipetting up and down twice. Be careful not to introduce any bubbles.

   IMPORTANT: Do not vortex the cells.
7. Centrifuge the tube at 300 x g for 2-3 minutes to pellet the cells.
8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in 10-15 mL of AC16 Expansion Medium.
10. Transfer the cell mixture to a T75 tissue culture flask.
11. Incubate the cells at 37°C in a humidified incubator with 5% CO_2.
12. The next day, exchange the medium with 10-15 mL of fresh AC16 Expansion Medium. Exchange with fresh medium every two to three days thereafter.
13. When the cells are approximately 90-95% confluent, they can be dissociated with Accutase (EMD Millipore Cat. No. SCR005) or trypsin-EDTA (EMD Millipore Cat. No. SM-2003-C) and further passaged or, alternatively, frozen for later use.

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Subculturing Cells

1. Carefully remove the medium from the T75 tissue culture flask containing the confluent layer of AC16 cells.
2. Apply 3-5 mL of Accutase or trypsin-EDTA solution and incubate in a 37°C incubator for 3-5 minutes.
3. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
4. Add 8 mL of AC16 Expansion Medium to the plate.
5. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
6. Centrifuge the tube at 300 x g for 3-5 minutes to pellet the cells.
7. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
8. Apply 2 mL of AC16 Expansion Medium to the conical tube and resuspend the cells thoroughly.

   IMPORTANT: Do not vortex the cells.
9. Count the number of cells using a hemocytometer.
10. Plate the cells to the desired density (typical split ratio is 1:5 - 1:6).

Cryopreservation of Cells

AC16 Human Cardiomyocyte Cell Line may be frozen in the expansion medium plus 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

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