Rev. 1

HPSC-derived Cardiomyocyte Cells (HPSC-CC)

Catalog #6240

Cell Specification

Human primary cardiomyocytes and cardiac tissue are superior modeling systems for heart disease studies, drug discovery and toxicity testing compared to animal cells or immortalized cell lines. Human adult primary cardiomyocytes are difficult to obtain due to limited availability of cardiac tissue and they do not typically proliferate in culture. The cardiomyocytes derived from human pluripotent stem cells (hPSC) can potentially provide an unlimited supply of cardiomyocytes for drug screening, toxicity testing and developmental studies.

HPSC-derived Cardiomyocyte Cells (HPSC-CC) of ScienCell Research Laboratories are differentiated from hPSC and enriched for contracting cells. Through the temporal modulation of canonical Wnt signaling and growth factor induction, monolayer-cultured hPSC are differentiated to contracting cardiomyocytes under serum-free and feeder-free conditions at high efficiency. HPSC-CC are cryopreserved at passage one after enrichment and delivered frozen. Each vial contains >1.5 x 10^6 cells in 1 ml volume. Cells are characterized by immunofluorescence with antibodies specific to alpha-actinin and sarcomeric tropomyosin. After reviving, HPSC-CC will begin contracting within 48 hours when cultured in Cardiomyocyte Growth Medium (CGM, Cat. #5901D). HPSC-CC are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi. HPSC-CC are guaranteed to further culture under the conditions provided by ScienCell Research Laboratories. *HPSC-CC are not recommended for expanding since the cells show limited proliferation capability in Cardiomyocyte Growth Medium (CGM, Cat. #5901D) in culture.*

Product Content

1 vial of frozen HPSC-CC (Cat. #6240, 1 mL, > 1.5 x 10^6 cells/vial)
50 mL Cardiomyocyte Plating Medium-basal (Cat. #5921)
1 mL 50x Cardiomyocyte Plating Medium Supplement (Cat. #5972)
250 mL Cardiomyocyte Growth Medium-basal (Cat. #5901D)
5 mL Cardiomyocyte Growth Medium Supplement (Cat. #5952)

Recommended Medium

It is recommended to use the provided Cardiomyocyte Plating Medium (CPM, Cat. #5921) for seeding HPSC-CC *in vitro*. It is recommended to use Cardiomyocyte Growth Medium (CGM, Cat. #5901D) or other preferred medium for the culturing of HPSC-CC *in vitro*.

Product Use

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

Storage
Upon receiving, directly and immediately transfer the cells from dry ice to liquid nitrogen and keep the cells in liquid nitrogen until they are needed for experiments.

Shipping
Dry ice.

Instructions for culturing cells

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

Note: HPSC-CC are very sensitive cells and they are not expected to proliferate many times in culture. Experiments should be well organized before thawing the cells. The following procedures are optimized for 6-well plates. Indicated volumes are for one well of a 6-well plate; please adjust volumes accordingly.

Initiating the culture:

Note: ScienCell's cells must be cultured in a 37ºC, 5% CO_2 incubator. Cells are only warranted if ScienCell media and reagents are used and the recommended protocols are followed.

1. Coat 3 wells of a 6-well plate with fibronectin (2 µg/cm^2). Dilute 58 µl of fibronectin stock solution (ScienCell, Cat. #8248) into 6 mL sterile Dulbecco's phosphate buffered saline, Ca^{++} and Mg^{++} free (DPBS, ScienCell, Cat. #0303) and mix well. Add 2 mL of diluted fibronectin per well and leave the vessel in a 37ºC incubator overnight.

   Note: Alternatively, you may use your preferred matrix for coating.

2. Prepare complete Cardiomyocyte Plating Medium (CPM): thaw the 50x supplement at room temperature; decontaminate the external surfaces of medium bottle and supplement tube with 70% ethanol and transfer them to sterile field. Aseptically open the supplement tube and add to the basal medium with a pipette. Rinse the tube with medium to recover the entire volume.
3. On the day of thawing cells, warm the plating medium to room temperature. Aliquot 10 mL of the plating medium into a 15 mL conical tube and leave it in the hood.
4. Before thawing cells, aspirate the fibronectin solution from the coating wells and add 1 mL of the plating medium to each well. Return the vessel to the incubator.
5. Take one vial of cardiomyocyte cells out of the liquid nitrogen. Immediately transfer the vial into a 37ºC water bath and gently swirl it for 90 seconds or until most of contents are thawed and only a small piece of ice remains.

   Note: The viability of the cells will decrease if the vial contents are completely thawed.

6. Immediately remove the vial from the water bath, 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 a 2 mL pipette, gently re-suspend the contents of the vial and transfer to the 15 mL conical tube containing 10 mL of the plating medium. Wash the emptied vial with 1 mL medium and combine with the cell suspension in the tube.

   Note: Minimize the time for step 5-6.

7. Centrifuge the tube at 1000x rpm for 5 minutes at room temperature.

8. Aspirate supernatant carefully. Be careful not to disturb the cell pellet.
9. Tighten the cap of the tube and loosen the cell pellet by tapping the bottom of the tube. Add 6 mL of the plating medium into the tube and invert the tube several times to mix well. If large visible cell pellets present, try to break them into small pieces by gently pipetting 2 - 3 times with a 5 mL pipette.
10. Bring the fibronectin coated plate to the hood. Add 2 mL of cell suspension into each well. Replace the cover and gently rock the vessel to distribute the cells evenly.
11. Return the culture vessel to the incubator.
12. For best results, do not disturb the culture for 48 hours after the culture has been initiated. Change the growth medium the third day to remove unattached cells, then every other day thereafter.

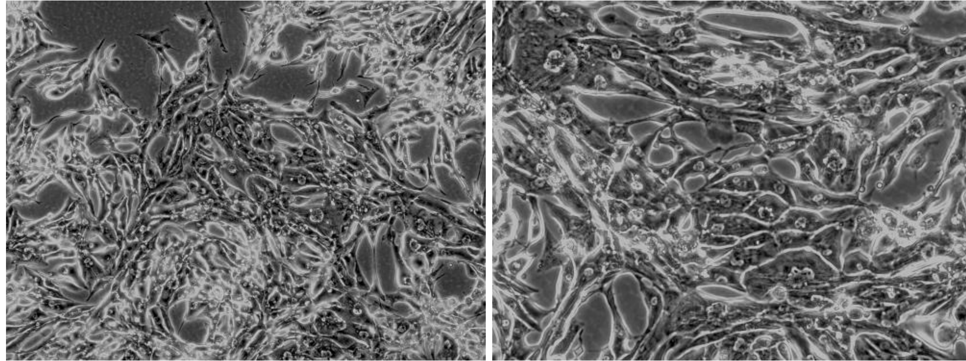
    We recommend using Cardiomyocyte Growth Medium (CGM, Cat. #5901D) to culture HPSC-CC. If needed, Cardiomyocyte Selective Medium (CSM, Cat. #5911) can be applied to further enrich the population and promote the maturation of cells. You may also use your preferred cardiomyocyte medium for the culture, although in this case we do not guarantee cell purity or that cells will contract.

    The revived HPSC-CC will begin contracting when cultured in CGM. The cells show limited proliferation capability in CGM.

Caution: Handling human-derived products is potentially biohazardous. Although each cell strain tests negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions must be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working with these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].

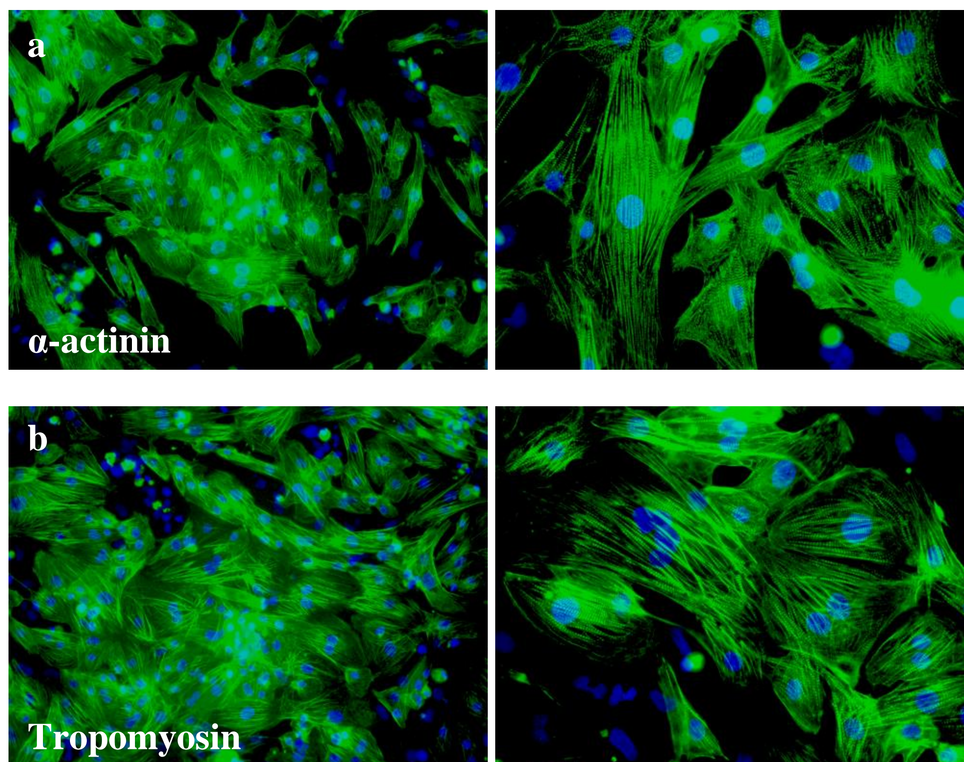
[1]. Grizzle, W. E., and Polt, S. S. (1988) "Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues." J Tissue Culture Methods. 11(4).

Figure 1. Human cardiomyocytes derived from human pluripotent stem cells at passage 1 before freezing



The monolayer cardiomyocytes are derived from human pluripotent stem cells, passaged once and cultured 6 days for enriching contracting cells before freezing. Left: 100x; right: 200x.

Figure 2. hPSC-derived cardiomyocyte cells express sarcomeric alpha-actinin and tropomyosin.



hPSC-derived cardiomyocyte cells are plated on matrigel-coated coverslips and cultured in CGM for characterization. Immunostaining for α-actinin (a, green) and tropomyosin (b, green) show sarcomere organization. Nuclei were stained with DAPI (blue).