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HPSC-derived Cardiomyocytes (H9-CM)

Catalog #6250

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. 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 Cardiomyocytes (H9-CM) from ScienCell Research Laboratories are differentiated from a pluripotent stem cell line (H9) and enriched for contracting cells. Through the temporal modulation of canonical Wnt signaling and growth factor induction, monolayer-cultured HPSCs are differentiated to contracting cardiomyocytes under serum-free and feeder-free conditions at high efficiency. H9-CM are cryopreserved at passage one after enrichment and delivered frozen. Each vial contains >1 x 10^6 cells in 1 ml volume. Cells are characterized by immunofluorescence with antibodies specific to α-Actinin and Sarcomeric Tropomyosin. H9-CM are negative for mycoplasma, bacteria, yeast and fungi. H9-CM are guaranteed to further culture under the conditions provided by ScienCell Research Laboratories. *H9-CM are not recommended for expanding since the cells show limited proliferation capability in culture.*

Recommended Medium

It is recommended to use Cardiac Myocyte Medium (CMM, Cat. #6201) for the culturing of H9-CM *in vitro*.

Product Content

Cat. #	# of vials	Product	Quantity	Storage
6250	1	HPSC-derived Cardiomyocytes (H9-CM)	1mL	Liquid Nitrogen
5921	1	Cardiomyocyte Plating Medium-basal	50 mL	4°C
5972	1	Cardiomyocyte Plating Medium Supplement (50X)	1mL	-20°C

Additional Materials Required (Not included)

Cat. #	Product
6201	Cardiac Myocyte Medium
8248	Bovine Plasma Fibronectin
0303	DPBS without Ca^{2+} and Mg^{2+}
5911	Cardiomyocyte Selective Medium (optional)
5901D	Cardiomyocyte Growth Medium (optional)

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Product Use
H9-CM 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 Cat. #6250 from dry ice to liquid nitrogen and keep the cells in liquid nitrogen until they are needed for experiments. Store Cat. #5921 at 4°C in the dark and Cat. #5972 at -20°C.

Shipping
Cat. #6250 and Cat. #5972 are shipped on dry ice. Cat. #5921 is shipped at room temperature.

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Instructions for culturing cells

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

***Note**:* H9-CM 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. Please adjust volumes accordingly if using a different plate type.

Initiating the culture:

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 37°C 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 1ml to the 50ml of 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 8 mL of the plating medium and transfer 7 mL of plating medium from 8 mL into a 15 mL conical tube and leave it in the hood.

4. Take one vial of cardiomyocyte cells out of the liquid nitrogen. Immediately transfer the vial into a 37°C water bath and gently swirl it 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.

5. 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 resuspend the contents of the vial. Transfer cell suspension to the 15 mL conical tube containing 7 mL of the plating medium prepared in Step 3. Wash the emptied vial with 1 mL medium and combine with the cell suspension in the tube.

   Note: Minimize the time for step 4-5.

6. Bring the fibronectin coated plate to the hood and aspirate the fibronectin from the well. Gently mix cell suspension with 5 mL pipette and add 3 mL of cell suspension into each well. Replace the cover and gently rock the vessel to distribute the cells evenly.

7. Return the culture vessel to the incubator.

8. 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.

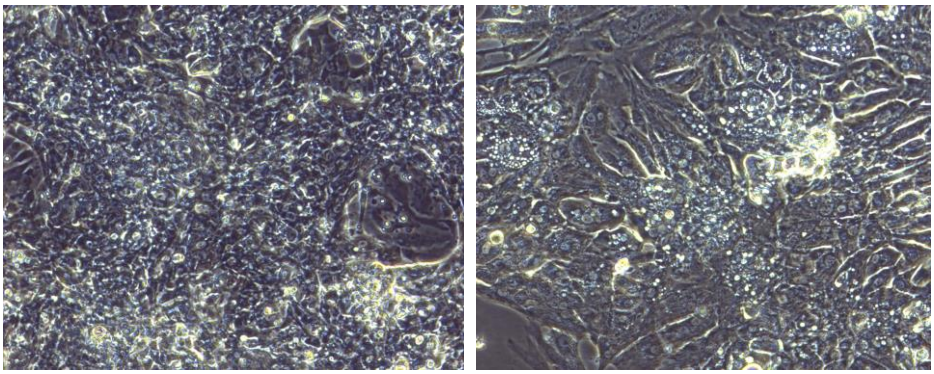
***Note**: We recommend using Cardiac Myocyte Medium (CMM, Cat. #6201) to culture HPSC-CM. If needed, Cardiomyocyte Selective Medium (CSM, Cat. #5911) can be applied to further enrich the population. You may also use your preferred cardiomyocyte medium for the culture.*

The revived HPSC-CM can restore contracting when culturing in CMM. If not, change the medium to Cardiomyocyte Growth Medium (CGM). The cells show limited proliferation capability in CMM.

Caution: Handling human-derived products is potentially biohazardous. 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].

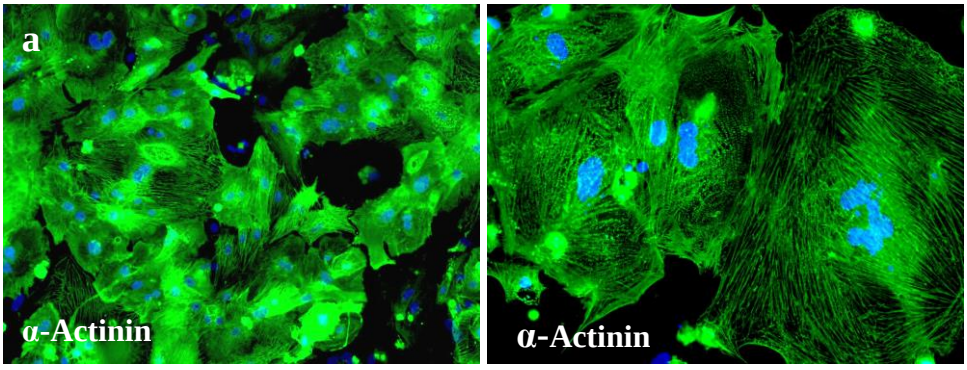
[1]. Grizzle, W. E., and Polt, S. S. (1988) "Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues." J Tissue Culture Methods. 11(4).

Figure 1. Phase contrast image of human cardiomyocytes derived from HPSCs.



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

Figure 2. HPSC-derived cardiomyocyte cells express α-Actinin and Sarcomeric Tropomyosin.



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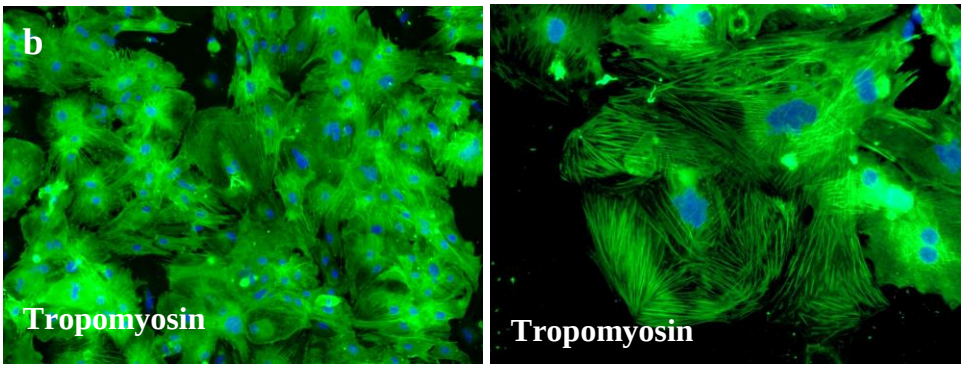

HPSC-derived cardiomyocyte cells are plated on fibronectin-coated coverslips and cultured in CMM for characterization. Immunostaining for α-Actinin (a, green) and Sarcomeric Tropomyosin (b, green) show sarcomere organization. Nuclei were stained with DAPI (blue). Left: 200x; right: 400x.