Human Pluripotent Stem Cell Cardiomyocyte Differentiation Kit (PSCCDK)

Catalog #5901

Introduction

Human pluripotent stem cells (hPSC), including embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC), possess the capability of differentiating into all derivatives of the three germ layers. Given that adult cardiomyocytes do not typically proliferate, hPSC can potentially provide an unlimited supply of cardiomyocytes. The cardiomyocyte derived from hPSC can be used for drug screening, toxicity testing, and developmental studies.

The spontaneous cardiac differentiation of hPSC is inefficient, with less than 1% of cardiomyocytes present in the culture. The differentiation process can be significantly enhanced by adding growth factors (BMP4, activin A, and FGF2 etc.), and small molecules, or using mouse END-2 cell-conditioned medium. The appropriate temporal addition of growth factors is also critic for cardiac differentiation. The Wnt signaling pathway is one of the key regulators in cardiac differentiation of hPSC. Modulating the Wnt signaling activity at different stages of differentiation is sufficient to drive hPSC to differentiate into cardiomyocytes.

ScienCell's Human Pluripotent Stem Cell Cardiomyocyte Differentiation Kit (PSCCDK) is specially designed to differentiate hPSC to cardiomyocytes at a high efficiency, combining growth factor induction with the temporal modulation of canonical Wnt signaling by small molecules. The kit efficiently converts monolayer-cultured pluripotent stem cells to contracting cardiomyocytes within 15 days under serum-free and feeder-free conditions. With the PSCCDK there is no need for the intermediate embryoid body formation. The kit contains 3 differentiation media, 1 basal medium, and 1 growth supplement. One kit is enough to differentiate 2 12-well plates of cells.

The PSCCDK kit is developed using hESC WA09 (H9) cultured in STEMium® medium (Cat. #5801). We consistently get a sheet of cardiomyocytes from H9 cells. Using the kit, we are also able to derive contracting clusters from a hiPSC line which is generated from human dermal fibroblasts using mRNA reprogramming technology. The hPSC-derived cardiomyocyte cells can be passaged and further enriched using our Cardiomyocyte Selective Medium (Cat. #5911).

Kit Components

Cat. #	# of vials	Reagent	Quantity	Storage
5901A	1	Cardiomyocyte Differentiation Medium A	50 ml	-20°C
5901B	1	Cardiomyocyte Differentiation Medium B	50 ml	-20°C
5901C	1	Cardiomyocyte Differentiation C	50 ml	-20°C
5901D	1	Cardiomyocyte Growth Medium	250 ml	4°C
5952	1	Cardiomyocyte Growth Medium Supplement	5 ml	-20°C

Note: One kit (#5901) is enough to differentiate two 12-well plates of cells.

Additional Materials Required

Product	**Vendor and Catalog Number**
BD Matrigel™ hESC-qualified matrix	BD Biosciences, Cat. #354277
StemDS® cell dissociation solution	ScienCell Research Laboratories, Cat. #5803
DPBS without Ca^{2+} and Mg^{2+}	ScienCell Research Laboratories, Cat. #0303
Y-27632 ROCK Inhibitor	Tocris Bioscience, Cat. #1254

Storage

Cardiomyocyte differentiation medium A, B, and C: store at -20°C; keep from light.
Cardiomyocyte growth medium: store at 4°C; keep from light.
Cardiomyocyte growth supplement: store at -20°C; keep from light.

Stability

Stable for six months if stored as directed.

Shipping

The medium A, B, C and the growth supplement are shipped on dry ice. The cardiomyocyte growth medium is shipped at 4°C.

Instructions for use

Controlled growth of hPSC in STEMium® medium:

Note: It is critical to maintain quality, undifferentiated pluripotent stem cells for differentiation. The cells should be passaged when cultures are approximately 80 - 90% confluent and sub-cultured every 4 - 5 days.

Note: hPSCs are maintained under feeder-free conditions. We suggest culturing the cells in STEMium® medium (Cat. #5801) to obtain the highest efficiency. Cells cultured in mTeSR1 or MEF-conditioned medium can also successfully differentiate into cardiomyocytes at an equal efficiency using this kit. Please refer to the STEMium® medium brochure for detailed instructions.

Note: The following procedures are optimized for 6-well plates. Indicated volumes are for one well of a 6-well plate; please adjust volumes accordingly.

1. Prepare BD Matrigel™-coated culture vessel in advance and warm to room temperature prior to use. For instructions on coating plates with BD Matrigel™, please refer to the manual.
2. Warm sufficient volumes of complete STEMium® medium (Cat. #5801, Cat. #5852) and StemDS® cell dissociation solution (Cat. #5803) for passaging.
3. Wash cells once with 2 mL of Ca^{2+}/Mg^{2+}-free phosphate-buffered saline (DPBS, Cat. #0303).
4. Aspirate DPBS and gently add 2 mL of StemDS® per well. Incubate at 37°C for 6 - 8 minutes. Colonies should remain adherent after incubation when viewed under a microscope.
5. Aspirate StemDS® completely.
6. Dislodge the cells from the plate by dispersing 2 mL of STEMium® onto the cells and pipetting up and down 1 - 3 times.

   It is best if a 5 mL serological pipette is used to loosen the colonies. If needed, use a p1000 tip to gently dislodge any large visible clumps. Optimal splitting ratio is 1:6 to 1:10.
7. Transfer the appropriate volume of cell suspension into a conical tube containing STEMium® medium. Mix well by inverting the tube several times.
8. Aspirate BD Matrigel™ from the plate and add 3 mL of cell suspension per well.
9. Distribute the cells evenly by gently rocking the plate several times. Incubate cells in a 37°C, 5% CO_2 with 95% humidity incubator.
10. Medium should be changed on a daily basis until the cells are ready for passaging and are about 80 - 90% confluent. Use a minimum of 3 mL of STEMium® medium per well of a 6-well plate.

Preparing monolayer cultures for cardiomyocyte differentiation:

Note: The following procedures are optimized for 12-well plates. Indicated volumes are for one well of a 12-well plate; please adjust volumes accordingly. For 6-well plates, use 3 mL of medium per well; for T-25 flasks, use 5 mL per flask

Note: When hPSC cultures are approximately 80 - 90% confluent, cells are ready for splitting and seeding onto 12-well plates for differentiation.

1. Prepare BD Matrigel™-coated 12-well plates in advance and warm to room temperature prior to use.
2. Warm sufficient volumes of culture medium and StemDS® cell dissociation solution (Cat. #5803) for splitting. Add Y-27632 ROCK inhibitor to the culture medium to a final concentration of 5 µM.
3. Wash cells once with 2 mL of DPBS.
4. Aspirate DPBS and gently add 2 mL of StemDS® per well. Incubate at 37°C for 6 - 8 minutes. Colonies should remain adherent after incubation when viewed under a microscope.
5. Aspirate StemDS®.
6. Dislodge cells from plate by dispersing 2 mL of culture medium onto the cells and pipetting up and down 1 - 3 times.
7. Count the number of live cells using a hemocytometer.
8. Plate the cells in the culture medium containing 5 µM Y-27632 ROCK Inhibitor. We strongly suggest optimizing the cell plating density in the first differentiation experiment. You may plate 0.05, 0.1, 0.25, 0.5, 0.75, 1.0, 1.25, and 1.5 million cells per well in a 12-well plate in 2 mL of culture medium per well. Once the optimal seeding density is identified, you can use it for the subsequent differentiation experiment.

   Note: If you use hESC H9 cells culturing in STEMium® medium for differentiation, we recommend seeding the cells at a density of 0.8 – 1.0 x 10^5 cells/well in 2 mL of medium for a 12-well plate, or 4.0 x 10^5 cells in 5 mL of medium for one T-25 flask.
9. Distribute the cells evenly by gently rocking the plate several times. Culture the cells in the incubator overnight.

   Note: Even distribution of the cells is crucial for high differentiation efficiency.

Differentiating monolayer hPSC to cardiomyocytes:

Note: The starting cell density is crucial for differentiation efficiency. You need to optimize the initial plating density for your specific culture condition.

Note: The following procedures are optimized for 12-well plates. Indicated volumes are for a single well of a 12-well plate; please adjust volumes accordingly.

1. **On Day 0**, thaw Cardiomyocyte Differentiation Medium A at room temperature.
2. Decontaminate the external surfaces of the medium bottle with 70% ethanol and transfer it to a sterile field.
3. Mix the medium well by pipetting up and down several times.
4. Aspirate the hPSC culture medium from the cells and add 2 mL of medium A. Record the time.
5. Culture the cells in a 37°C, 5% CO2 with 95% humidity incubator for 24 hours.
6. **On Day 1**, thaw medium B at room temperature and mix well. Exactly **<u>24 hours</u>** after adding medium A, change medium and apply 2 mL of medium B to the cells.

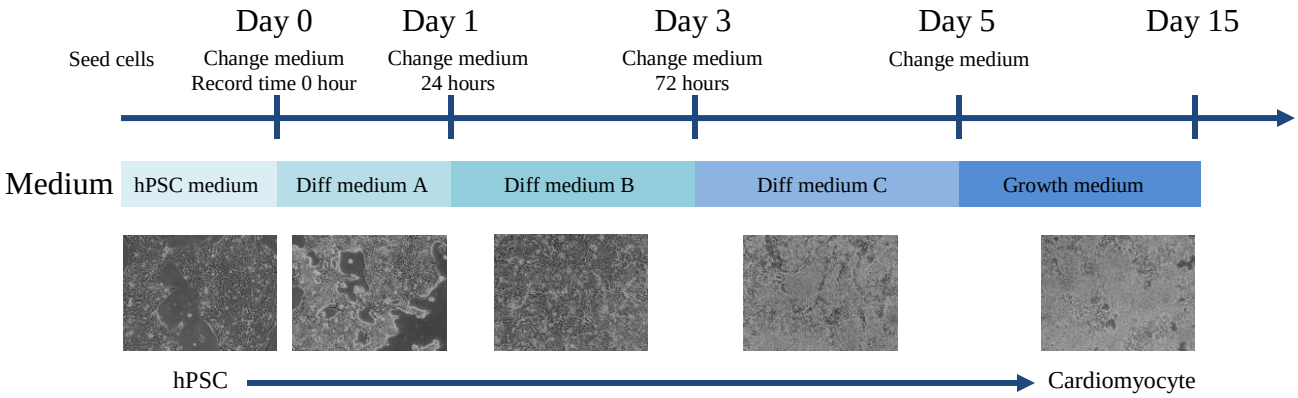
   Note: the time point is critical here. Incubate the cells with medium A for no more than 24 hours.

   Note: Some cell death is expected after 24 hours in medium A, however, if you lose >90% of cells, the experiment is likely to fail. To prevent this from happening, increase the initial cell plating density or shorten medium A induction time.
7. **On Day 3**, thaw medium C at room temperature and mix well.
8. **<u>72 hours</u>** after the initial time recorded, aspirate medium B from the cells and add 2 mL per well of medium C.
9. **On Day 5**, thaw Cardiomyocyte Growth Supplement and warm Cardiomyocyte Growth Medium at room temperature to prepare complete Cardiomyocyte Growth Medium. Make sure the growth supplement is completely dissolved before adding it to the basal medium.
10. Add the growth supplement to the basal medium using sterile techniques and mix well.
11. Aspirate medium C from the cells and apply 2 mL of the complete growth medium per well. Change the medium every other day afterwards until spontaneously contracting cardiomyocytes appear. Contracting cells could be observed as early as days 7 - 10 and keep increasing until day 15.

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 WE, Polt S. (1988) "Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues." *J Tissue Culture Methods*. 11: 191-9

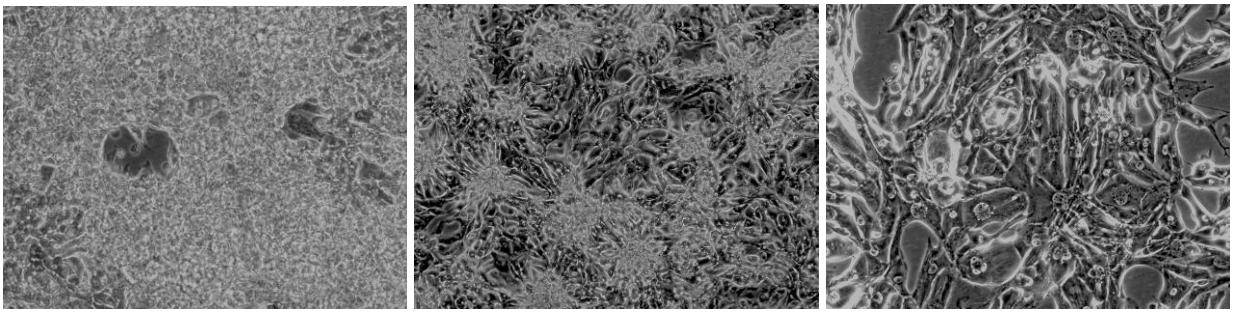
Schematic of cardiomyocyte differentiation kit procedure:



Troubleshooting Table

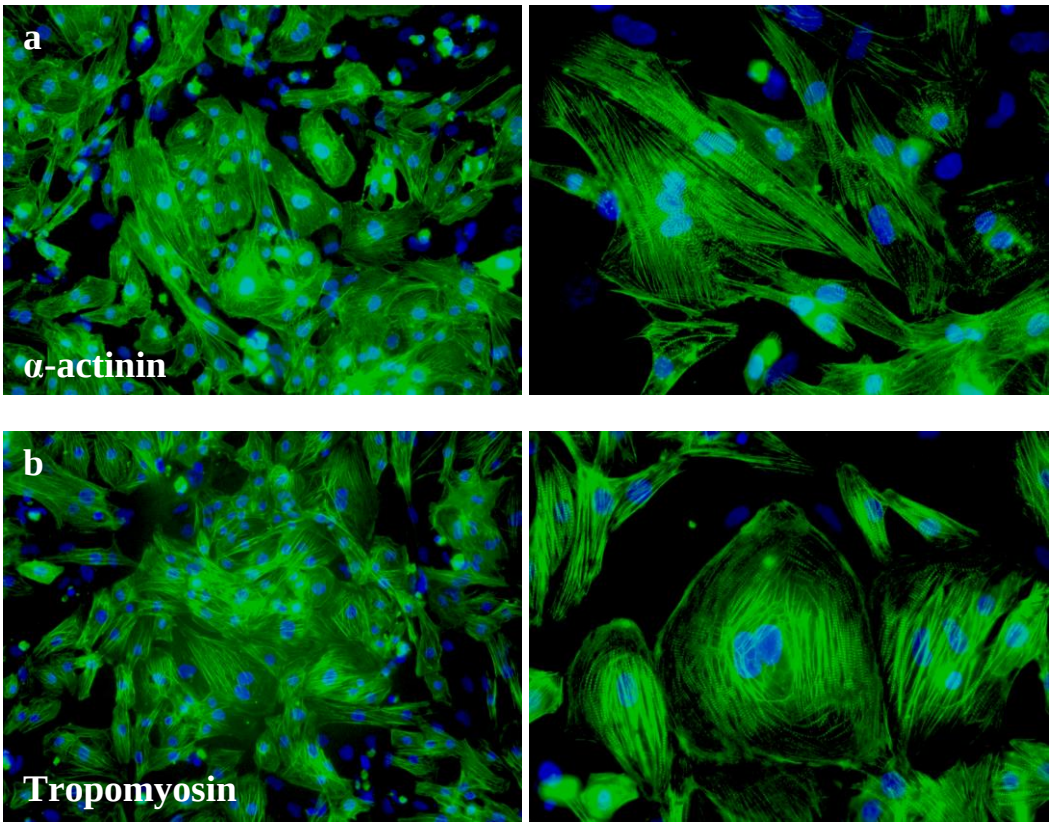
Problem	Suggestions
Poor attachment of hPSC in Matrigel™-coated plates	Seed cells in medium containing 5 µM ROCK inhibitor Y-27632.
Dramatic cell death or detachment after culturing in medium A	Increase cell plating density; incubate cells with medium A for no more than 24 hours or shorten the medium A induction time.
No contracting cells or low differentiation efficiency	Optimize initial cell plating density; start with quality undifferentiated hPSC; it is critical to induce the cells with medium A for 24 hours

Figure 1. Cardiomyocytes derived from hPSC using Cardiomyocyte Differentiation Kit



Using Cardiomyocyte Differentiation Kit (PSCCDK), hPSC can be differentiated to cardiomyocyte at high efficiency. Left: a contracting layer of cardiomyocytes on day 13 of passage 0 (P0); middle & right: the contracting cardiomyocyte at P1.

Figure 2. Cardiomyocytes derived from hPSC expressing sarcomeric alpha-actinin and tropomyosin.



hPSC-derived cardiomyocytes were replated on the Matrigel-coated coverslips on day 16. Immunostaining for α-actinin (a, green) and tropomyosin (b, green) show sarcomere organization. Nuclei were stained with DAPI (blue).