CELL
APPLICATIONS, INC.

General Instructions for Use

Induction of Human Cardiomyocytes (i-HCm) from Human induced Pluripotent Stem Cells (HiPSC)

Be sure to wear face protection mask and gloves when retrieving cryovials from the liquid nitrogen storage tank. The dramatic temperature change from the tank to the room could cause any trapped liquid nitrogen in the cryovials to burst and cause injury.

Open all the packages immediately upon arrival and examine each component for shipping damage. Notify Cell Applications, Inc. or your distributor immediately if there is any problem.

I. STORAGE

A. i-HUMAN CARDIOMYOCYTE DIFFERENTIATION MEDIUM

Store at 4°C in the dark immediately upon arrival and used within 4 weeks.

i-HCm Differentiation Medium 1 (017D1-30)
i-HCm Differentiation Medium 2 (017D2-30)
i-HCm Differentiation Medium 3 (017D3-100)

B. i-HUMAN CARDIOMYOCYTE SELECTION MEDIUM (019-50)

Store i-HCm Selection Medium 1 at 4°C in the dark immediately upon arrival and used within 4 weeks.

C. i-HUMAN CARDIOMYOCYTE MAINTENANCE MEDIUM (021-30)

Store i-HCm Maintenance Medium at -20°C in the dark immediately upon arrival and should be kept at 4°C after thawing and used within 4 weeks.

Medium and Reagents not included in the Kit:

HiPSC GROWTH MEDIUM (015-500)
HiPSC COATING SOLUTION (126-100)
HiPSC DISSOCIATION KIT (091K)
i-HCm PLATING MEDIUM (021P-10)
ROCK Inhibitor Y27632: R&D Systems (1254)
100 X Stock is prepared:
Dissolve as 0.32 mg/ml in nanopure water
Sterile filtered through 0.2 filter
TryplE Express: ThermoFisher (12604-013)

II. PREPARATION FOR CULTURING

1. Make sure the Class II Biological Safety Cabinet, with HEPA filtered laminar airflow, is in proper working condition.
2. Clean the Biological Safety Cabinet with 70% alcohol to ensure it is sterile.
3. Turn the Biological Safety Cabinet blower on for 10 min. before cell culture work.
4. Make sure all serological pipettes, pipette tips and reagent solutions are sterile.
5. Follow the standard sterilization technique and safety rules:
   a. Do not pipette with mouth.
   b. Always wear gloves and safety glasses when working with human cells even though all the strains have been tested negative for HIV, Hepatitis B and Hepatitis C.
   c. Handle all cell culture work in a sterile hood.

III. i-HCm INDUCTION FROM HiPSC

The instruction below is for 6 well plate format. Please change the cell number and media volume for other sizes in Appendix.

****Prior to differentiation, HiPSC have to be passaged at least 2 times after initial thawing. *Only use robust culture of HiPSC that should be 80-90% confluent by day 3 or 4 during subculture.***

A. PREPARING 6-well CULTURE PLATE FOR SUBCULTURING HiPSC

1. Take the HiPSC Coating Solution and HiPSC Growth Medium kit from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile hood.

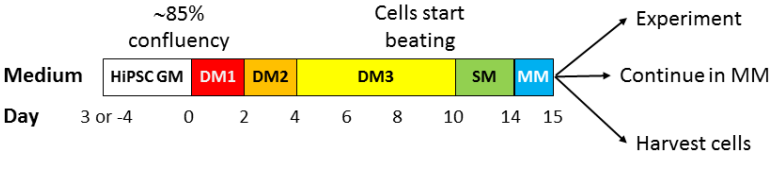

Figure 1: HiPSC to i-HCm schematic using the Cardiomyocyte Differentiation Media Kit

2. Prepare one 6 well plate for seeding HiPSC by pipetting 1 ml of HiPSC Coating Solution to each well. See Appendix for volume used in other tissue culture wares.
3. Incubate coated 6 well plate at 37ºC for a minimum of 30 minutes.

B. SEED HiPSC for INDUCTION

1. When HiPSC culture is 80-90% confluent, cells are ready for subculture in 1:15 ratio (range from 1:12 to 1:20 depending on the initial confluency and speed of growth)
2. Remove Coating Solution from each well.
3. Seed HiPSC into Matrigel coated well.
4. By day 3 to 4, the cells should reach 75-85% confluency are ready for i-HCm induction.

C. i-HCm INDUCTION

Use timeline figure for scheduling.
Pre-equilibrate all the Medium for 2 hours in 37ºC, 5% CO_2 humidified incubator prior to use.
When change medium, aspirate the old medium completely from the wells one by one. Do not let cells in the well dried up.

1. On day 0 of induction (day 3 or 4 after subculture in Section IIIB, Step 3) take out the 6 well plate from the incubator, aspirate the HiPSC media completely and add 2 ml pre-equilibrated i-HCm Differentiation Medium 1 (017D1-30) to each well.
2. Put the plate back to the incubator for 2 days.
3. On day 2 of induction, take out the 6 well plate from the incubator and aspirate the i-HCm Differentiation Medium 1 (017D1-30) completely from the wells, and add 2 ml i-HCm Differentiation Medium 2 (017D2-30) to each well.
4. Put the plate back to the incubator for 2 days.
5. On day 4 of induction, take out the 6 well plate from the incubator and aspirate the i-HCm Differentiation Medium 2 (017D2-30) completely from the wells, and add 2 ml i-HCm Differentiation Medium 3 (017D3-30) to each well.
6. Put the plate back to the incubator for 2 days.
7. Change the i-HCm Differentiation Medium 3 (017D3-100) on day 6 and day 8 (the cells should start beating by day 8 or 9)
8. On day 10 of induction, take out the 6 well plate from the incubator and aspirate the i-HCm Differentiation Medium 3 (017D3-100) completely from the wells.
9. Add 2 ml i-HCm Selection Medium (017S-50) to each well.
10. Put the plate back to the incubator for 2 days.
11. Change the i-HCm Selection Medium (019-50) on day 12.
12. On day 14, selection ends. Take out the 6 well plate from the incubator and aspirate the i-HCm selection Medium (019-50) completely from the wells.
13. Add 2 ml i-HCm Maintenance Medium (021-100) to each well.

D. OPTIONS FOR i-HCm on Day 15:

1. Maintain i-HCm: Change Maintenance Medium (021-100) every other day. i-HCm can be cultured for additional 15 more days.
2. Subculture i-HCm:
   a. Aspirate i-HCm Maintenance Medium (021-100) from wells.
   b. Wash the cells three times with PBS.
   c. Add 1 ml TrypLE-Express and incubate at the 37°C incubator for 5 min.
   d. Pipet up and down with a 1 ml P-1000 aerosol tip set at 950 µl to break cell clumps. Avoid forming bubbles.
   e. Place the plate in the incubator for another 5 min at 37°C.
   f. Take the plate out, pipet up and down with a 1 ml P-1000 tip Aerosol tip set at 950 ml to break cell clumps.
   g. Transfer the cells to a 15 ml conical tube containing 10 ml i-HCm Maintenance Medium (021-100) and mix gently.
   h. Pass the cell through a 100 µm cell strainer into a 50 ml conical tube.
   i. Centrifuge the i-HCm at 300 x g for 5 min.
   j. Take the supernatant off and resuspend cells gently in 2 ml i-HCm i-HCm Plating Medium (021P-10).
   k. Count cells.
   l. Seed the cells in a Matrigel coated culture plate (as in Sec IIIB) with seeding density of 250,000 cells/cm^2.
   m. On 2nd day of seeding i-HCm, change the medium to i-HCm Maintenance Medium (021-100).
   n. Continue culturing the i-HCm for approx. 30 days with i-HCm Maintenance Medium (021-100). Change medium every other day (cells should start beating between days 5-7).

Appendix 1: Common tissue cultureware formats with corresponding working volumes.

TC Vessel	Volume per Well			
	HiPSC Coating Solution	HiPSC Growth Medium	PBS	HiPSC Dissociation Solution
T-25 Flask	3 ml	5 ml	5 ml	2.5 ml
6 cm Dish	3 ml	4 ml	4 ml	2 ml
10 cm Dish	6 ml	10 ml	10 ml	5 ml
15 cm Dish	15 ml	20 ml	20 ml	15 ml
6-Well Plate	1 ml	2 ml	2 ml	1 ml
12-Well Plate	0.5 ml	1 ml	1 ml	0.5 ml
24-Well Plate	0.25 ml	0.5 ml	0.5 ml	0.25 ml

5820 Oberlin Drive, #101
San Diego, CA 92121
Tel: (858) 453-0848 Toll-Free: (800) 645-0848 Fax: (858) 453-2862
Email: info@cellapplications.com Web: www.cellapplications.com