

General Instructions for Culturing

iPSC-Derived Human Cardiomyocytes (i-HCm)

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. i-HCm STORAGE

A. CRYOPRESERVED VIALS (i357-20)

Store the cryovials in a liquid nitrogen storage tank immediately upon arrival.

One cryovial of i-HCm is for seeding 5 wells of 24 well plate or 10 wells of 48 well plate (~200,000 cells/cm²).

B. i-HCm COATING SOLUTION KIT (038K)

Diluent Store at 4°C

Coating Stock store at -20°C immediately upon arrival

C. i-HCm PLATING MEDIUM (021P-10)

Store at 4°C in the dark immediately upon arrival.

D. i-HCm MAINTENANCE MEDIUM (021-50)

Store at 4°C in the dark immediately upon arrival.

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 standard sterilization technique and safety rules:
 - a. Do not pipette with mouth.
 - b. Always wear lab coat, gloves, and safety glasses.
 - c. Handle all cell culture work in a sterile hood.

III. CULTURING i-HCm

A. PREPARATION OF i-HCm COATING SOLUTION

1. Thaw the iHCm Coating Stock for 1 hour at 4°C prior to preparing i-HCm Coating Solution on the day of seeding i-HCm.

2. Transfer thawed stock in i-HCm Coating Stock vial to the diluent to make i-HCm Coating Solution. Wash Stock vial with i-HCm Coating Solution and transfer the wash to i-HCm Coating solution.
i-HCm Coating Solution has to be used in the same day.

B. PREPARATION OF CULTURE WARE FOR CULTURING

1. Add 0.25 ml of the Coating Solution to each well of a 24 well plate or 0.125 ml to each well of a 48 well plate and gently distribute to obtain a homogeneous coating surface.
3. Incubate for 1h at 37°C. Do not allow wells to dry
4. i-HCm Maintenance and Plating Medium should be thawed overnight at 4°C prior to use.
5. Shelf life of Plating Medium after thaw is 3 months. Shelf life of Maintenance Medium should be used within 4 weeks after thaw.
6. Equilibrate required amount of Plating Medium at room temperature before thawing i-HCm.

B. THAWING AND PLATING i-HCm

*** Pre-wet the tips with medium to reduce cells sticking to tips and avoid the loss of cells.**

1. Remove the cryopreserved vial of i-HCm from the liquid nitrogen storage tank using proper protection for your eyes and hands.
2. Turn the vial cap a quarter turn to release any liquid nitrogen that may be trapped in the threads, then re-tighten the cap.
3. Thaw the cells quickly by placing the lower half of the vial in a 37°C water bath for 90 seconds. Take the vial out of the water bath when only small amount of ice remaining in the vial. Do not let cells thaw completely.
4. Decontaminate the vial exterior with 70% alcohol in a sterile Biological Safety Cabinet.
5. Remove the vial cap carefully. Do not touch the rim of the vial.
6. Resuspend the cells in the vial by gently pipetting the cells once with a pre-wetted 1 ml aerosol pipette tip set at 950 µl. Be careful not to pipette too vigorously.
7. Slowly transfer the cell suspension from the cryovial into a 15 ml conical tube.

8. Rinse the empty cryovial with 1 ml of room temperature Plating Medium to recover any residual cells from the vial. Transfer the 1 ml plating medium rinse from the cryovial drop wise to the 15 ml conical tube containing i-HCm cell suspension.
9. Slowly add 10 ml of i-HCm Plating Medium to the cells in 15 ml conical tube while swirling the tube to mix.
10. Gently mix the contents of the 15 ml conical tube by inverting 2-3 times. Avoid vigorous shaking.
11. Centrifuge at 200 x g for 5 minutes to pellet the cells.
12. Aspirate the supernatant from the tube without disturbing the cell pellet.
13. Flick the tip of the conical tube with your finger to loosen the cell pellet gently.
14. **Resuspend the i-HCm in 5 ml Plating Medium (for 5 wells of a 24 well plate, 1 ml per well) or 6 ml Plating Medium (for 10 wells of a 48 well plate, 0.6 ml per well) and gently pipette to mix well.**
15. Aspirate i-HCm Coating Solution from the well of the plate.
16. Add the cell suspension to the designated wells of the plate of choice.
17. Incubate the i-HCm culture in a 37°C, 5% CO₂ humidified incubator for 48 hours.
18. 48 hours post plating of iHCm, gently aspirate the Plating Medium and replace with appropriate volumes of the Maintenance Medium (1 ml per well of 24 well plate and 0.6 ml per well of 48 well plate).
19. Replace the Maintenance Medium every other day.
20. The cardiomyocytes should start beating between day 4 to day 6.
21. Beating i-HCm can be maintained in the Maintenance Medium for up to 3 weeks.

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