

General Instructions for Use

Human Cardiomyocytes (i-HCm) Generated from Human induced Pluripotent Stem Cells

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-HCm (i357-20)
Store at liquid nitrogen immediately upon arrival.
- B. i-HCm MAINTENANCE MEDIUM (021-50)
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.
- C. i-HCm PLATING MEDIUM (021P-20)
Store i-HCm Plating Medium at -20°C in the dark immediately upon arrival and should be kept at 4°C after thawing and used within 4 weeks.
- D. i-HCm COATING SOLUTION (126-05)
Store i-HCm Coating Solution at -20°C in the dark immediately upon arrival and should be kept at 4°C after thawing and used within 4 weeks.

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. CULTURING i-HCm

The instruction below is for 48 well plate format. Please change the cell number and media volume for other sizes in Appendix.

- A. PREPARING 48-well CULTURE PLATE FOR i-HCm CULTURE
1. Take i-HCm Coating Solution, i-HCm Plating Medium, and i-HCm Maintenance Medium from the freezer and allow to thaw at 4°C overnight. Decontaminate the bottles with 70% alcohol in a sterile hood.
 2. Prepare 10 wells of one 48 well plate for seeding i-HCm by pipetting 200 ul of i-HCm Coating Solution to each well. See Appendix for volume used in other tissue culture wares.
 3. Incubate the coated 48 well plate at 37°C for 1h.
- C. THAWING AND PLATING i-HCm
- **Seeding density of i-HCm: ~ 200,000/cm²**
1. Prepare a 15 ml conical tube by pipetting 9 ml of iHCm Plating Medium to the conical tube for use in Step 8.
 2. Remove the cryopreserved vial of i-HCm from the liquid nitrogen storage tank using proper protection for your eyes and hands.
 3. Turn the vial cap a quarter turn to release any liquid nitrogen that may be trapped in the threads, then re-tighten the cap and bury the cryovial in dry ice.
 4. Thaw the cells quickly by placing the lower half of the vial in a 37°C water bath and watch the vial closely during the thawing process. This usually takes about 90 sec.
 5. Take the vial out of the water bath when only small amount of ice left in the vial. Do not let cells thaw completely.
 6. Decontaminate the vial exterior with 70% alcohol in a sterile Biological Safety Cabinet.
 7. Remove the vial cap carefully. Do not touch the rim of the cap or the vial.

8. Transfer the cell suspension drop wise to the 15 ml conical tube containing 9 ml i-HCm Plating Medium prepared in Step 1. Mix gently.
9. Centrifuge cells at 200g for 5 minutes. Remove supernatant carefully by aspiration without disturbing the cell pellet.
10. Re-suspend i-HCm in 7.5 ml i-HCm Plating Medium.
11. Aspirate i-HCm Coating Solution from the 10 wells of 48 well plate prepared in Section III A step 3.
12. Add 0.75 ml of i-HCm suspension to each well of 48 well plate. Rock gently to evenly distribute the cells.
13. Place the 48 well plate in a 37°C, 5% CO₂ humidified incubator. For best results, do not disturb the culture for 48 hours after plating.
14. After 48 hours, slowly aspirate the Plating Medium and add 0.75 ml i-HCm Maintenance Medium.
15. Change i-HCm Maintenance Medium every other day.
16. The cells should start beating from day 4-5 after plating.

Appendix 1: Common tissue culture ware formats with corresponding working volumes.

TC Vessel	Volume per Well			
	i-HCm Coating Solution	i-HCm Plating Medium		i-HCm maintenance medium
6-Well Plate	1 ml	3 ml		3 ml
12-Well Plate	0.5 ml	2 ml		2 ml
24-Well Plate	0.25 ml	1 ml		1 ml
48-Well Plate	0.2 ml	0.75 ml		0.75 ml