Materials and Equipment

Materials

- Cardiomyocytes (AC008-F)
- Cardiomyocyte Cellutions Maintenance Medium (C-MAIN)
- TGF-β-1 (1 ng/ml, concentration in C-MAIN, to be added – not supplied)
- Collagen coating solution (150 µg/ml)
- 1× PBS (Ca^{2+}/Mg^{2+}free)
- Fetal Bovine Serum (FBS)
- 15 ml, 50 ml conical centrifuge tubes
- Cell culture vessels

Equipment

- Biosafety Cabinet
- Water Bath 37°C
- Centrifuge
- Incubator, 37°C, 5% CO2
- Pipet-Aid
- Pipettes

Procedure

1.	Immediately Upon Delivery
	• Remove vial from shipping container and check that it is still frozen—if thawed, contact DV Biologics.
	• Transfer frozen vial immediately to liquid nitrogen until you are ready to thaw and begin cell culture.
	• Prior to thawing cells, make sure to have all required reagents and materials ready to use.
	• Coat cell culture vessels with 150 µg/ml collagen coating solution (0.2 ml/cm^2).
	  ○ Incubate at room temperature for 2hrs.
	  ○ Aspirate collagen coating solution.
	  ○ Wash the culture vessel with PBS.

2.	Thawing Cells
	• Perform and maintain all cell culture using aseptic techniques.
	• Prepare C-MAIN medium. After mixing, store complete medium at 4°C for a maximum of 30 days.
	• Aliquot and warm only media required for use that day at 37°C.
	• To the C-MAIN medium for use that day, add fresh TGF-β-1 (1 ng/mL).
	• Thaw cells rapidly and with agitation (See How to Thaw Cells).
	  ○ Slowly dilute thawed cells with 3- to 10-fold excess cold FBS (or complete C-MAIN medium).
	  ○ Centrifuge at 400 g for 5 minutes.
	• Remove supernatant. Disperse cell pellet in 1-5 ml fresh 37°C warm C-MAIN medium with TGF-β-1.
	• Count Cells (See How to Count Cells).

3.	Plating and Maintaining Cells
	• Carefully add cell suspension to collagen-coated culture vessel.
	  ○ Plate cells at a minimum cell seeding density of 5.0 x 10^4 cells/cm^2.
	  ○ Leave cells undisturbed for a minimum of 2 days.
	• Feed cells on the third day by completely replacing the medium with fresh C-MAIN with TGF-β-1. NOTE: There may be many unattached cells. You may collect these, centrifuge and reseed.
	• Maintain cells in C-MAIN with TGF-β-1, feeding every 2-3 days, and observe cells daily.
	  ○ Cells should be fed and maintained in culture for a minimum of 10 days prior to use.
	  ○ Cells should be kept confluent for optimal results.