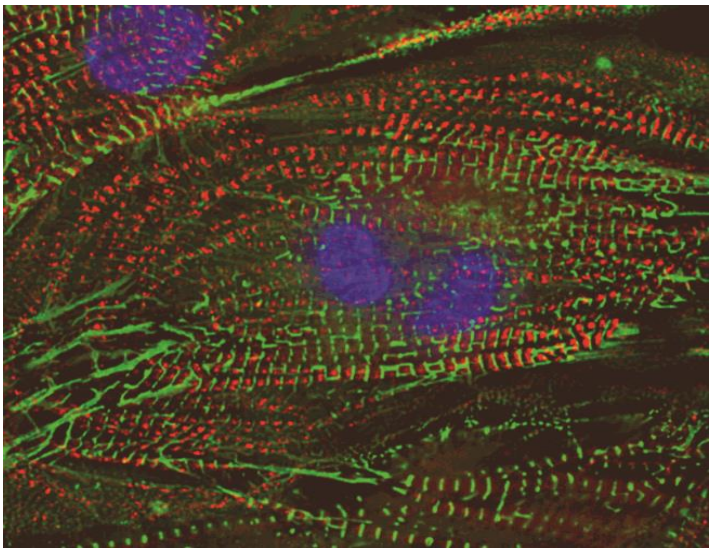
Cytiva™ Plus Cardiomyocytes

A relevant human model of cardiotoxicity

Product Instructions

Codes:	ES-7001	≥1.0 × 10^6 (1,000,000) viable cells
	ES-7002	≥3.5 × 10^6 (3,500,000) viable cells



ASCENDANCE™

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http://www.esibio.com

2. Handling

Cytiva Plus Cardiomyocytes are cryopreserved in a solution containing dimethyl sulphoxide (DMSO). A Material Safety Data Sheet (MSDS) for the DMSO is available upon request by phone ((510)-522-2856) or by email at techsupport@esibio.com.

2.1. Safety warnings and precautions

Warning: ***For research use only.*** Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals. All chemicals should be considered as potentially hazardous. We therefore recommend that this product be handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

2.2. Storage

Cytiva Plus Cardiomyocyte product codes (ES-7001) and (ES-7002) are supplied in 1 ml cryovials of ≥1×10^6 (1,000,000) viable cells (1.6×10^6 total cells) and ≥3.5×10^6 (3,500,000) viable cells (5×10^6 total cells) respectively. Cells are cryopreserved in 10% DMSO and 90% fetal bovine serum. Upon receipt, the frozen vial(s) of cells should immediately be removed from the outer packaging and transferred to the vapor phase of a liquid nitrogen storage unit at -140°C.

2.3. Expiration

Cells are stable for at least 6 months when stored in liquid nitrogen (vapor phase). Cells can be maintained for at least 7 days once recovered into culture.

2.4. Packaging

Cytiva Plus Cardiomyocytes are provided as cryopreserved single cell suspensions in 1 mL cryovials.

3. Introduction

Cytiva Plus Cardiomyocytes are human cardiomyocytes derived from the NIH approved stem cell line NIH hESC-10-0061 (WA07 (H7)) and have been extensively characterized and functionally verified by flow cytometry, sub-cellular imaging and electrophysiology. Cytiva Plus Cardiomyocytes are supplied cryopreserved in a ready to use format.

4. Components and other materials required

4.1. Components

- Cytiva Plus Cardiomyocytes
- Certificate of Analysis including product specifications
- User manual

4.2. Materials to be supplied by user

Product	Supplier/Product code
15 mL Centrifuge tubes	Corning 430052
50 mL Centrifuge tubes	Corning 430290
Serological pipettes – 5 mL	Corning 4487
Serological pipettes – 10 mL	Corning 4488
Serological pipettes – 25 mL	Corning 4489
500 mL Cellulose acetate filter units	Corning 430769
12- or 48-well plate for Multi-Electrode Array (MEA) applications	e.g. Axion BioSystems M768-GLx or M768-KAP-48
Multi-well cell plates for High Content Analysis (HCA) and Ca^{2+} transient applications	Various, e.g. 96-well plate Greiner µClear® 781091
Multi-well plates for impedance applications	e.g. ACEA E-plate Cardio 96
Coverslips - electrophysiological applications*	VWR 631-0149
1.5 mL tubes	Corning 430290
Sterile bottle (e.g. 30 or 60 mL)	NALG2019-0030/0060
RPMI 1640 + Glutamine	Gibco 21875034
D-PBS	Sigma D8537
B27 (50×)	Gibco 17504-004
Fibronectin	BD Biosciences 354008
FBS	Gibco 26140-079
Matrigel®	Becton Dickinson 356231
KnockOut® D-MEM (KO-DMEM)	Gibco 10829-018
Sterile distilled water	Fresenius Kabi 22-96-985

* –not available in North America. Standard glass coverslips of approximately 13 mm diameter and 0.13 mm thickness are suitable.

4.3. Equipment needed

- Adjustable pipettes and tips
- Liquid nitrogen vapor store
- Biosafety cabinet
- Ice bucket with dry ice
- Cryovial rack
- Vacuum pump and line
- Hemocytometer or automated cell counter
- 37°C water bath
- Centrifuge

Application dependent:

- Patch clamp system
- Sub-cellular imaging system for High Content Analysis
- Multi-Electrode Array system
- Impedance system
- Calcium transient system

5. Protocols for Multi-Electrode Arrays (MEA)

5.1. Preparation of MEA coated plates (Time 4 hours)

Consumables

- FBS (at 4°C)
- Sterile distilled H2O
- Fibronectin (1 mg, human, BD Biosciences 354008) (at 4°C)
- 50 ml tube
- D-PBS

Equipment

- MEA plate(s) (e.g. Axion BioSystems 12-well or 48-well plates)*
- 20 µL pipette & sterile 1-20 µL tips
- 1,000 µL pipette & sterile 1-1,000 µL tips

***Note:** Approximate number of MEA plates that can be used with different vial sizes of Cytiva Plus Cardiomyocytes (assuming $6.00x10^4$ cells/well; > 70% viability).

Cytiva Plus Cardiomyocyte Vial Size	**Approx. Number of MEA Plates**		
	6-well	12-well	48-well
ES-7001 - $1.0x10^6$ viable cells/vial	2.8	1.4	0.3
ES-7002 - $3.5x10^6$ viable cells/vial	9.7	4.9	1.2

Protocol

Perform the following steps aseptically inside the biosafety cabinet (BSC). The first step in preparing an MEA plate for use is to ensure that the surface is hydrophilic. The surface of a new MEA plate is hydrophobic, and even hydrophilic MEA wells tend to become hydrophobic again during storage. A hydrophobic surface will prevent attachment and growth of the (hydrophilic) cells. FBS treatment renders the surface hydrophilic.

1. Place a 4 µL bead of FBS over the recording electrode area of each well of the MEA plate (see Fig 1). **Note:** If a bead fails to form, ignore that recording site.
2. For a 12 well MEA plate, add 500 µL of sterile distilled H_2O to the gaps between the wells to prevent the evaporation of the bead of FBS (see Fig 1). For a 48 well MEA plate add 200 µL of sterile distilled H_2O to the gaps between the wells.
3. Put the lid on the MEA plate and incubate the MEA plate for 1.5 hours at room temperature.
4. Prepare a 1 mg/mL solution of fibronectin by adding 1 mL sterile distilled H_2O to the 1 mg fibronectin.
5. Take 12.5 µL of this 1 mg/mL fibronectin solution and add it to 987.5 µL D-PBS in a 50 mL tube for a final concentration of 12.5 µg/mL fibronectin.
6. Aspirate the FBS bead from each well of the MEA plate (use a pipette set to dispense 8 µL) and **immediately** replace with a 4 µL bead of 12.5 µg/mL fibronectin solution over the recording electrode area.

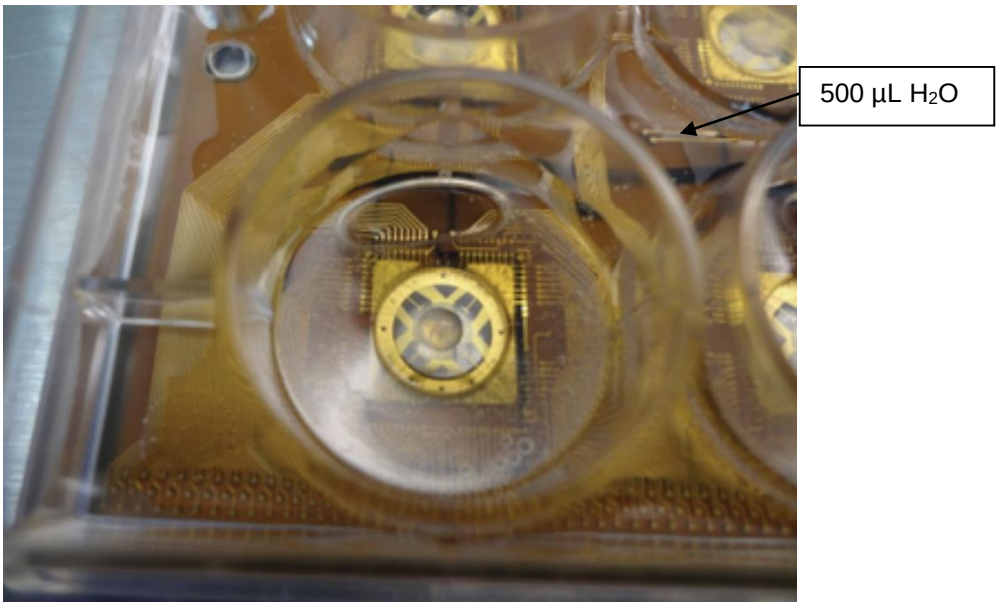


Figure 1. MEA plate showing location of sterile water addition to the inter-well space to prevent evaporation of the FBS and fibronectin drop.

5.2. Preparation of RPMI 1640/B27 medium for MEA (Time 30 minutes)

Consumables

- RPMI 1640 + Glutamine medium, 500 mL (at 4°C)
- B27® supplement, 10 mL (at 4°C)
- 10 mL sterile serological pipette

Equipment

- 500 mL 0.22 µm cellulose acetate filter unit (Corning 430769)
- Vacuum line
- Pipette gun

Protocol

During the 2-hour fibronectin incubation step, prepare medium.

> **Note:** Medium may be prepared immediately before thawing Cytiva Plus Cardiomyocytes or prepared and stored at 2–8°C and used within one week of preparation.

1. Thaw frozen 10 mL B27 supplement vial(s) in a 37°C water bath for 10 minutes. Do not incubate at 37°C or expose to light for extended periods of time. Perform the following steps aseptically inside the biosafety cabinet (BSC).
2. Wipe the required number of RPMI 1640 + Glutamine medium bottle(s) and B27 Supplement vial(s) with 70% isopropanol and transfer to a BSC.
3. Place the filtration unit into the BSC.
4. Using a 10 mL sterile serological pipette, add 10 mL of B27 supplement to a 500 mL bottle of RPMI1640 + Glutamine medium. Swirl bottle several times to mix.
5. Carefully transfer the medium and supplement into the reservoir of the filter unit.
6. Place the lid on the filter unit.
7. Connect the filter unit to a vacuum source.
8. When filtration is complete, disconnect the vacuum source.
9. Detach the upper reservoir of the filtration unit.
10. Place sterile cap on the bottle portion of the filter unit.

> **Note:** Store the medium at 2–8°C. Use within one week of preparation. Avoid repeated warming of the RPMI 1640/ B27 medium. Warm only the required volume of medium to complete the task.

5.3. Thawing Cytiva Plus Cardiomyocytes for MEA (Time 30 minutes)

Consumables

- 3.5×10^6 vial of Cytiva Plus Cardiomyocytes (ES-7001)
- RPMI 1640/B27 medium (filter sterilized)
- 50 mL tube
- 10 mL sterile serological pipette

Equipment

- Ice bucket with dry ice
- Cryovial rack
- 37°C water bath
- Centrifuge
- 1,000 µL pipette & sterile 1-1,000 µL tips
- Pipette gun

Protocol

During the 2-hour fibronectin incubation step, perform the following steps aseptically inside the biosafety cabinet (BSC).

1. Remove the cryovial from cryostorage and place onto dry ice until ready to thaw.
2. Thaw cell suspension in a 37°C water bath with gentle agitation until ice crystals just disappear.

Note: Take care not to immerse the whole cryovial into the water bath. Avoid extended incubation at 37°C.

3. Wipe the outside of the cryovial with 70% isopropanol and transfer to BSC.
4. Carefully transfer the cell suspension into a sterile 50 mL centrifuge tube using a 1,000 µL pipette.
5. Rinse the inside of the cryovial with 1 mL of room temperature RPMI 1640/B27 and combine with the cell suspension drop-wise with gentle mixing.
6. Slowly (over the course of 2 minutes) add 8 mL of RPMI 1640/B27 to the 50 mL centrifuge tube.
7. Centrifuge at 300 g for 5 minutes at 20°C.
8. Carefully, using a 10 mL stripette, remove 8 mLs of the supernatant. Remove a further 1 mL with a 1,000 µL pipette taking care not to disturb the cell pellet. Re-suspend the cells in the residual liquid (approx. 1 mL) with gentle agitation.

5.4. Determining Post-Thaw Cell Viability of Cytiva Plus Cardiomyocytes for MEA (Time 30 minutes)

During the 2-hour fibronectin incubation step, determine the viable cell number and viable cell density using preferred method of choice. We use a NucleoCounter NC-100 (ChemoMetec) cell counter, the method for which is outlined below. However, this protocol could be adapted for other commercial cell counters.

Consumables

- RPMI 1640/B27 medium (filter sterilized)
- 4×1.5 mL tubes
- 4×NucleoCassettes™ (ChemoMetec)
- Reagent A100 (ChemoMetec)
- Reagent B (ChemoMetec)

Equipment

- 100 µL pipette & sterile 1-200 µL tips
- 1,000 µL pipette & sterile 1-1,000 µL tips
- NucleoCounter NC-100 (ChemoMetec)

Protocol

Perform the following steps aseptically inside the biosafety cabinet (BSC).

1. Determine viable cell number using the NucleoCounter NC-100 (ChemoMetec). To do this, transfer 40 µL of the cell suspension into a 1.5 mL tube. Add to the tube 360 µL of RPMI 1640/B27 medium (i.e. now a 1:10 dilution). Gently agitate to achieve an even cell suspension.
2. The remaining steps can be performed outside of the BSC.
3. Transfer 100 µL of 1:10 diluted cell suspension into each of three more 1.5 mL tubes.
4. Use 100 µL of 1:10 diluted cell suspension sample to calculate the number of Non-Viable Cells/mL (i.e. directly load the sample into a NucleoCassette).
5. Repeat step 4 for a second time, and calculate the average result.
6. Use another 1×100 µL of 1:10 diluted cell suspension sample to calculate the Total Number of Cells/mL. To do this, add 100 µL of Reagent A100, and then 100 µL of Reagent B, to the 100 µL of 1:10 diluted cell suspension sample. Mix by pipetting, then load into a NucleoCassette.
7. Repeat step 6 for a second time, and calculate the average result.
8. Determine the total Volume of Cell Suspension.

Note: We use a 1,000 µL pipette to establish this volume.

9. Calculate the number of viable cells in the cell suspension:

- Viable cells/mL = 10 × [(3 × Total Number of Cells/mL)-(Non-Viable Cells/mL)]
- Total number of viable cells = Viable cells/mL × Volume of Cell Suspension (mL)

Worked example for a typical 3.5×10^6 vial of Cytiva Plus Cardiomyocytes:

Total Number of Cells/mL determined in step 6 = 1.83×10^5

Non-Viable Cells/mL determined in step 5 = 1.64×10^5

Viable cells/mL = 10 × [(3 × 1.83×10^5) - (1.64×10^5)] = 3.85×10^6 cells/mL

Total number of viable cells = 3.85×10^6 cells/mL × 1 mL = 3.85×10^6 cells

Note: There are a total of 5.5×10^6 cells in a 3.5×10^6 vial of Cytiva Plus Cardiomyocytes. We routinely record a value of >70% post thaw cell viability (i.e. 3.85×10^6 viable cells).

5.5. Seeding Cytiva Plus Cardiomyocytes onto MEA plates (Time 3 hours)

Cells should be seeded at a density of 6×10^4 viable cells in 4 µL (i.e. 1.5×10^7 viable cells/mL) over the recording electrode area.

Note: Assuming the volume of the cell suspension is ~1 mL, and the number of viable cells is 3.85×10^6, the current concentration is ~3.85×10^6 viable cells/mL. Consequently, the next step involves centrifuging and re-suspending the cells from section 5.4 to achieve the appropriate cell concentration.

Consumables

- RPMI 1640/B27 medium (filter sterilized)
- 50 mL tube
- 60 mL bottle

Equipment

- Fibronectin coated well MEA plate
- 20 µL pipette & sterile 1-20 µL tips
- 200 µL pipette & sterile 1-200 µL tips
- 1,000 µL pipette & sterile 1-1,000 µL tips

Protocol

Perform the following steps aseptically inside the biosafety cabinet (BSC).

1. Warm 25 mL RPMI 1640/B27 medium in a 60 mL bottle (sealed) in a 37°C water bath.
2. Centrifuge the cell suspension in a 50 mL tube at 300 g for 5 minutes at 20°C.
3. Carefully, using a 1,000 µL pipette, remove the supernatant.

4. Re-suspend the cells in the residual liquid with gentle agitation.
5. Determine the total volume of the cell suspension.

Note: We use a 100 µL pipette to establish this volume.

6. Dilute the cell suspension to 1.5×10^7 viable cells/mL using warm RPMI 1640/B27 medium.

$$\text{Required volume (mL)} = \frac{\text{Viable cells}}{1.5\times10^7 \text{ viable cells/mL}}$$

Note: Assuming number of viable cells is 3.85×10^6, the final volume should be 260 µL.

7. Remove fibronectin-coated well MEA plate from incubator.
8. Aspirate the fibronectin bead from each well of the MEA plate (use a pipette set to dispense 8 µL) and **immediately** replace with a 4 µL bead of Cytiva Plus Cardiomyocyte suspension (1.5×10^7 viable cells/mL) over the recording electrode area (see Fig 2).

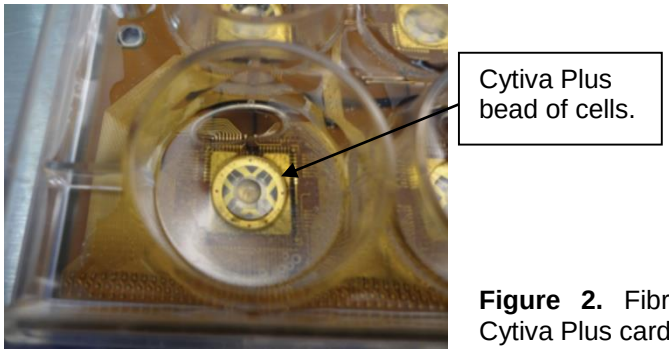


Figure 2. Fibronectin bead replaced with Cytiva Plus cardiomyocyte bead.

9. After seeding all wells, put the lid on the tissue culture dish and incubate the MEA plate in a standard cell culture incubator at 37°C for 2–3 hours. The sterile distilled water in the gaps between the wells will prevent the evaporation of the bead of cell suspension.
10. After 2–3 hours, gently add 500 µL of warm RPMI 1640/B27 medium to the 'corner' of each well of the 12-well MEA plate or 150 µL to the 'corner' of each well of the 48-well MEA plate. Pipette against the side of the well so as not to disturb the plated cells.
11. Gently add another 500 µL of warm RPMI 1640/B27 medium to the 'corner' of each well of the 12-well MEA plate or 150 µL to the 'corner' of each well of the 48-well MEA plate.

12. Gently add another 1,000 µL of warm RPMI 1640/B27 medium to the 'corner' of each well of the 12-well MEA plate (i.e. total well volume is now 2 mL) or another 150 µL to the 'corner' of each well of the 48-well MEA plate (i.e. total well volume is now 450 µL).
13. Aspirate the 500 µL of sterile distilled H_2O from the gaps between the wells in the 12-well MEA plate or 200 µL of sterile distilled H_2O from the gaps between the wells in the 48-well MEA plate.
14. Confirm cell attachment at this point by observing the MEA wells under the microscope if using a transparent plate (see Fig 3A and 3B).
15. Incubate in a standard cell culture incubator at 37°C, 5% CO_2.

Figure 3A – Wells containing Cytiva Plus Cardiomyocytes and media.

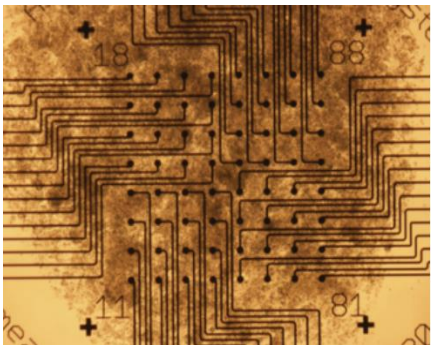


Figure 3B –Cytiva Plus Cardiomyocytes as viewed under a microscope, 5 days after seeding.

5.6. Media change on day 4 post-thaw (Time 20 minutes)

1. Warm 30 mL RPMI 1640/B27 medium in a 60 mL bottle in a 37°C water bath.
2. Perform the following steps aseptically inside the biosafety cabinet (BSC).
3. For the 12-well MEA plate, using a 1,000 µL pipette, carefully aspirate 1,000 µL of media from each well of the 12-well MEA plate, then gently add 1,000 µL of warm RPMI 1640/B27 medium to the 'corner' of each well of the 12-well MEA plate, to give a total volume of 2 mL.
4. For the 48-well plate, using a 1,000 µL pipette, carefully aspirate 250 µL of media from each well of the 48-well MEA plate then using a 200 µL pipette, gently add 200 µL of warm RPMI 1640/B27 medium to the 'corner' of each well of the 48-well MEA plate, to give a total well volume of 400 µL.
5. Perform MEA recordings 5–7 days after plating onto the MEA plate. User must determine the optimal culture time for this application.

6. Protocols for High Content Analysis (HCA)

6.1. Preparation of cell culture plates for HCA (Time 2½ hours)

Consumables

- Sterile distilled H_2O
- Fibronectin (1 mg, human, BD Biosciences 354008) (at 4°C)
- 50 mL sterile tube
- D-PBS (sigma D8537)

Equipment

- 384–well cell culture plate (e.g. Greiner µClear 781091)*
- 96-well cell culture plate (e.g. Greiner µClear 655090)*
- 20 µL pipette & sterile 1–40 µL tips
- 1,000 µL pipette & sterile 1–1,000 µL tips

***Note:** Cytiva Plus Cardiomyocyte - ES-7002 ($3.5x10^6$ viable cells/vial) provides sufficient cardiomyocytes **for 1 96-well plate ($3.0x10^4$ cells/well) or 1 384-well plate ($9.0x10^3$ cells/well).**

Protocol

Perform the following steps aseptically inside the biosafety cabinet (BSC).

1. Prepare a 1 mg/mL solution of fibronectin by adding 1 mL sterile distilled water to the 1 mg fibronectin.
2. Take 125 µL of this 1 mg/mL fibronectin solution and add it to 9,875 µL D-PBS in a 50 mL tube for a final concentration of 12.5 µg/mL fibronectin. Add 100 µL of 12.5 µg/mL fibronectin solution to each well of the 96–well cell culture plate, or 30 µL to each well of a 384–well cell culture plate.
3. Put the lid on the cell culture plate and incubate for 2 hours in a standard cell culture incubator at 37°C, 5% CO_2.

6.2. Preparation of RPMI 1640/B27 medium for HCA (Time 20 minutes)

Consumables

- RPMI 1640 + Glutamine medium, 500 mL (at 4°C)
- B27 supplement, 10 mL (at -20°C)
- 10 mL sterile serological pipette

Equipment

- 500 mL 0.22 µm cellulose acetate filter unit (Corning 430769)
- Vacuum line
- Pipette gun

Protocol
During the 2-hour fibronectin incubation step, prepare medium.

> **Note:** Medium may be prepared immediately before thawing Cytiva Plus Cardiomyocytes or prepared and stored at 2–8°C and used within one week.

1. Thaw frozen 10 mL B27 supplement vial(s) for 10 minutes in a 37°C water bath. Do not incubate at 37°C or expose to light for extended periods of time. Perform the following steps aseptically inside the biosafety cabinet (BSC).
2. Wipe the required number of RPMI 1640 + Glutamine medium bottle(s) and B27 Supplement vial(s) with 70% isopropanol and transfer to a BSC.
3. Place the filtration unit into the BSC.
4. Using a 10 mL sterile serological pipette, add 10 mL of B27 supplement to 500 mL bottle of RPMI 1640 + Glutamine medium. Swirl bottle several times to mix.
5. Carefully pour 510 mL of the RPMI 1640/B27 medium into the reservoir of the filtration unit.
6. Place the lid on the filter unit.
7. Connect the filter unit to a vacuum source.
8. When filtration is complete, disconnect the vacuum source.
9. Detach the upper reservoir of the filtration unit.
10. Place sterile cap on the bottle portion of the filter unit.

Note: Store the medium at 2–8°C. Use within one week of preparation. Avoid repeated warming of the RPMI 1640/ B27 medium. Warm only the required volume of medium to complete the task.

6.3. Thawing Cytiva Plus Cardiomyocytes for HCA (Time 30 minutes)

Consumables

- Cytiva Plus Cardiomyocytes- ES-7002
- RPMI 1640/B27 medium (filter sterilized)
- 50 mL sterile tube
- 10 mL sterile serological pipette

Equipment

- Ice bucket with dry-ice
- Cryovial rack
- 37°C water bath
- Centrifuge
- 1000 µL pipette & sterile 1-1000 µL tips
- Pipette gun

Protocol
During the 2-hour fibronectin incubation step, perform the following steps aseptically inside the biosafety cabinet (BSC).

1. Remove the cryovial from the cryostore and place onto dry-ice until ready to thaw.
2. Thaw cell suspension in a 37°C water bath with gentle agitation until ice crystals just disappear.

Note: Take care not to immerse the whole cryovial into the water bath. Avoid extended incubation at 37°C.

3. Wipe the outside of the cryovial with 70% isopropanol and transfer to BSC.
4. Carefully transfer the cell suspension into a sterile 50 mL centrifuge tube using a 1,000 µL pipette.
5. Rinse the inside of the cryovial with 1 mL of room temperature RPMI 1640/B27 and combine with the cell suspension drop-wise with gentle mixing.
6. Slowly (over the course of 2 minutes) add 8 mL of RPMI 1640/B27 to the 50 mL centrifuge tube.
7. Centrifuge at 300 g for 5 minutes at 20°C.
8. Carefully, using a 10 mL stripette, remove 8 mLs of the supernatant. Remove a further 1 mL with a 1,000 µL pipette taking care not to disturb the cell pellet. Re-suspend the cells in the residual liquid (approx. 1 mL) with gentle agitation.

6.4. Determining Post-thaw Cell Viability of Cytiva Plus Cardiomyocytes for HCA (Time 30 minutes)

During the 2-hour fibronectin incubation step, determine the viable cell number and viable cell density using preferred method of choice. We use a NucleoCounter NC-100 (ChemoMetec) cell counter, the method for which is outlined below. However, this protocol could be adapted for other commercial cell counters.

Consumables

- RPMI 1640/B27 medium (filter sterilized)
- 4×1.5 mL tubes
- 4×NucleoCassettes™ (ChemoMetec)
- Reagent A100 (ChemoMetec)
- Reagent B (ChemoMetec)

Equipment

- 100 µL pipette & sterile 1-200 µL tips
- 1,000 µL pipette & sterile 1-1,000 µL tips
- NucleoCounter NC-100 (ChemoMetec)

Protocol

Perform the following steps aseptically inside the biosafety cabinet (BSC).

1. Determine viable cell number using the NucleoCounter NC-100 (ChemoMetec). To do this, transfer 40 µL of the cell suspension into a 1.5 mL tube. Add to the tube 360 µL of RPMI 1640/B27 medium (i.e. now a 1:10 dilution). Gently agitate to achieve an even cell suspension.
2. The remaining steps can be performed outside of the BSC.
3. Transfer 100 µL of 1:10 diluted cell suspension into each of three more 1.5 mL tubes.
4. Use a 100 µL of 1:10 diluted cell suspension sample to calculate the number of Non-Viable Cells/mL (i.e. directly load the sample into a NucleoCassette).
5. Repeat step 4 for a second time, and calculate the average result.
6. Use another 1×100 µL of 1:10 diluted cell suspension sample to calculate the Total Number of Cells/mL. To do this, add 100 µL of Reagent A100, and then 100 µL of Reagent B, to the 100 µL of 1:10 diluted cell suspension sample. Mix by pipetting, then load into a NucleoCassette.
7. Repeat step 6 for a second time, and calculate the average result.
8. Determine the total Volume of Cell Suspension.

Note: We use a 1,000 µL pipette to establish this volume.

9. Calculate the number of viable cells in the cell suspension:

- Viable cells/mL = 10 × [(3 × Total Number of Cells/mL)-(Non-Viable Cells/mL)]
- Total number of viable cells = Viable cells/mL × Volume of Cell Suspension (mL)

Worked example for a typical 3.5×10^6 vial of Cytiva Plus Cardiomyocytes:

Total Number of Cells/mL determined in step 6 = 1.83×10^5

Non-Viable Cells/mL determined in step 5 = 1.64×10^5

Viable cells/mL = 10 × [(3 × 1.83×10^5) - (1.64×10^5)] = 3.85×10^6 cells/mL

Total number of viable cells = 3.85×10^6 cells/mL × 1 mL = 3.85×10^6 cells

Note: There are a total of 5.5×10^6 cells in a 3.5×10^6 vial of Cytiva Plus Cardiomyocytes and we routinely record a value of >70% post thaw cell viability (i.e. 3.85×10^6 viable cells).

6.5. Seeding Cytiva Plus Cardiomyocytes into cell culture plates for HCA (Time 30 minutes)

Cells should be seeded at a density of 3.6×10^4 viable cells in 200 µL RPMI 1640/B27 per well of a 96–well cell culture plate, or 9×10^3 viable cells in 50 µL RPMI 1640/B27 per well of a 384–well cell culture plate (i.e. 1.8×10^5 viable cells/mL).

Note: Assuming the volume of the cell suspension is ~1 mL, and the number of viable cells is 3.85×10^6, the current concentration is ~3.85×10^6 viable cells/mL. Consequently, the next step involves diluting the cell suspension to achieve the appropriate cell concentration.

Consumables

- RPMI 1640/B27 medium (filter sterilized)
- 60 mL bottle

Equipment

- Fibronectin coated cell culture plate
- 200 µL pipette & sterile 1-200 µL tip

Protocol

Perform the following steps aseptically inside the biosafety cabinet (BSC).

1. Warm 25 mL RPMI 1640/B27 medium in a sterile 60 mL bottle (sealed) in a 37°C water bath.
2. Dilute the cell suspension to 1.8×10^5 viable cells/mL using warm RPMI 1640/B27 medium.

$$\text{Required volume (mL)} = \frac{\text{Viable cells}}{1.8\times10^5 \text{ viable cells/mL}}$$

Example: A total viable count of 3.85×10^6 cells would be divided by 1.8×10^5 viable cells/mL to obtain a final required volume of 21.4 mL.

3. Remove fibronectin-coated cell culture plate from the incubator.
4. Aspirate the fibronectin from each well of the cell culture and **immediately** replace with 200 µL of Cytiva Plus Cardiomyocyte suspension (i.e. 1.8×10^5 viable cells/mL) per well of a 96-well culture plate, or 50 µL per well of a 384-well culture plate.
5. After seeding all the required wells, put the lid on the cell culture plate and incubate in a standard cell culture incubator at 37°C.

6.6. Media change on day 4 post-thaw (Time 20 minutes)

1. Warm 30 ml RPMI 1640/B27 medium in a 60 ml bottle (sealed) in a 37°C water bath.
2. Perform the following steps aseptically inside the biosafety cabinet (BSC).
3. After 96 hours, flick the media out of the plate onto clean tissues and replace with 40 µL of warmed RPMI 1640/B27 medium into each well so as not to disturb the seeded cells. Incubate plate at 37°C, 5% CO2.
4. Repeat a media change at day 7 replacing the seeded medium with fresh pre-warmed RPMI 1640/B27 medium.
5. Perform HCA 7–8 days after seeding in cell culture plates. It is the responsibility of the user to determine the optimal culture time for this application.

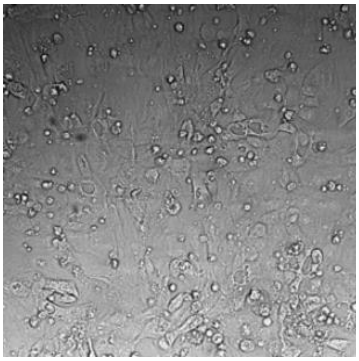


Figure 4 – Bright field image of Cytiva Plus Cardiomyocytes seeded in microplates for HCA at 8 days post thaw.

7. Protocols for Manual Patch Clamp (MPC)

7.1. Sterilization of glass coverslips (Time 4 hours)

Consumables

- Borosilicate glass coverslips (13 mm)
- Isopropanol
- 24-well cell culture plate
- 50 mL sterile tube

Equipment

- Sterile forceps

Protocol

Perform the following steps aseptically inside the biosafety cabinet (BSC).

1. Sterilize the coverslips by soaking in isopropanol for a minimum of 2 hours in a sealed sterile 50 mL tube. Ensure complete wetting of both sides of each coverslip.
2. Using sterile forceps, remove a coverslip from the isopropanol and shake the excess isopropanol from the coverslip. Place the coverslip into the well of a sterile 24-well plastic cell culture plate.
3. Repeat steps 1–3 for the number of wells required.
4. Let the coverslips air-dry in a BSC for a minimum of 2 hours.

7.2. Preparation of 1:2 diluted Matrigel aliquots (10 minutes with O/N thaw)

Consumables

- Matrigel (BD Biosciences 356231, -20°C)
- KO-DMEM (2–8°C)
- 10 mL sterile serological pipette (2–8°C)
- 10×50 mL sterile tubes (2–8°C)

Equipment

- Pipette gun

Protocol

Do not allow the Matrigel solution to reach room temperature. Keep the solution, pipettes, and KO-DMEM cold at all stages of handling. Avoid repeated freeze-thawing of diluted Matrigel aliquots. Perform the following steps aseptically inside the BSC.

1. Slowly thaw Matrigel at 2–8°C **overnight** to avoid the formation of a gel.
2. Cool a sterile 10 mL serological pipette by drawing and releasing 10 mL of cold KO-DMEM into the pipette repeatedly without removing the pipette from the bottle of KO-DMEM.
3. Add 10 mL of cold KO-DMEM to the vial containing 10 mL Matrigel.
4. Working quickly, mix the Matrigel and KO-DMEM with a 10 mL pipette, avoiding the formation of bubbles.
5. Aliquot 2 mL of diluted Matrigel into pre-chilled sterile 50 mL tube; store at -20°C until required. Diluted Matrigel solution is stable for 3 months when stored at -20°C.

7.3. Preparation of 1:30 diluted Matrigel (Time 10 minutes)

Consumables
- 2 mL 1:2 diluted Matrigel (-20°C)
- KO-DMEM (2–8°C)
- 5 mL sterile serological pipette (2–8°C)
- 25 mL sterile serological pipette (2–8°C)

Equipment
- Pipette gun

Protocol
Do not allow the Matrigel solution to reach room temperature. Keep the solution and pipettes cold at all stages of handling. Perform the following steps aseptically inside the biosafety cabinet (BSC).

1. Slowly thaw a 2 mL 1:2 diluted Matrigel aliquot prepared in step 7.2 at 4°C for at least 2 hours to avoid the formation of a gel. Once thawed, transfer to the BSC.
2. Cool a sterile 5 mL pipette by drawing 5 mL of cold KO-DMEM into the pipette.
3. Dilute the 2 mL 1:2 diluted Matrigel aliquot with 5 mL cold KO-DMEM. Carefully mix Matrigel solution, avoiding the formation of bubbles.
4. Cool a sterile 25 mL pipette by drawing 25 mL of cold KO-DMEM into the pipette.
5. Add a further 23 mL cold KO-DMEM (for a final dilution of 1:30). Carefully mix Matrigel solution, avoiding the formation of bubbles.

7.4. Preparation of Matrigel-coated coverslips (Time 10 minutes)

Consumables
- 30 mL 1:30 diluted Matrigel (4°C)
- 24-well cell culture plate containing glass coverslips
- KO-DMEM (2–8°C)
- 10 mL sterile serological pipette (2–8°C)
- 10 × 50 mL tubes

Equipment
- Pipette gun

Protocol
Do not allow the Matrigel solution to reach room temperature. Keep the solution, pipettes and KO-DMEM cold at all stages. Avoid repeated freeze-thawing of diluted Matrigel aliquots. Perform the following steps aseptically inside the biosafety cabinet (BSC).

1. Add 400 µL of 1:30 diluted Matrigel to each well of the 24-well cell culture plate containing a glass coverslip.
2. Incubate the cell culture plate overnight at 2–8°C before use.
3. Matrigel coated vessels are stable for 10 days when stored at 2–8°C.

7.5. Preparation of RPMI 1640/B27 medium for MPC (Time 20 minutes)

Consumables

- RPMI 1640 + Glutamine medium, 500 mL (at 4°C)
- B27 supplement, 10 mL (at 4°C)
- 10 mL sterile serological pipette

Equipment

- 500 mL 0.22 µm cellulose acetate filter unit (Corning 430769)
- Vacuum line
- Pipette gun

Protocol

Perform the following steps aseptically inside the biosafety cabinet (BSC).

1. Using a 10 mL sterile serological pipette, add 10 mL of B27 supplement to 500 mL bottle of RPMI 1640 + Glutamine medium. Swirl bottle several times to mix.
2. Place the filtration unit into the BSC.
3. Carefully pour 510 mL of the RPMI/B27 medium into the reservoir of the filtration unit.
4. Place the lid on the filter unit.
5. Connect the filter unit to a vacuum source.
6. When filtration is complete, disconnect the vacuum source.
7. Detach the upper reservoir of the filtration unit.
8. Place sterile cap on the bottle portion of the filter unit.

Note: Store the medium at 2–8°C. Use within one week of preparation. Avoid repeated warming of the RPMI 1640/B27 medium. Warm only the required volume of medium to complete the task.

7.6. Thawing Cytiva Plus Cardiomyocytes for MPC (Time 30 minutes)

Consumables

- 24-well cell culture plate containing Matrigel-coated coverslips (2–8°C)
- Cytiva Plus Cardiomyocytes (ES-7001 - $1.0x10^6$ cells/vial)
- RPMI 1640/B27 medium (filter sterilized)
- 50 mL sterile tube
- 10 mL sterile serological pipette

Equipment

- Ice bucket with dry-ice
- Cryovial rack
- 37°C water bath
- Centrifuge
- 1000 µL pipette & sterile 1–1000 µL tips
- Pipette gun

Protocol

Perform the following steps aseptically inside the biosafety cabinet (BSC).

1. Before thawing the cryovial of cells, take the 24-well cell culture plate containing the Matrigel-coated coverslips out of 2–8°C storage. Leave the 24-well cell culture plate at room temperature for 1 hour before seeding the cells.
2. Remove the cryovial from the cryostore and place onto dry-ice until ready to thaw.
3. Thaw cell suspension in a 37°C water bath with gentle agitation until ice crystals just disappear.

Note: Take care not to immerse the whole cryovial into the water bath. Avoid extended incubation at 37°C.

4. Wipe the outside of the cryovial with 70% isopropanol and transfer to BSC.
5. Carefully transfer the cell suspension into a sterile 50 mL centrifuge tube using a 1,000 µL pipette.
6. Rinse the inside of the cryovial with 1 mL of room temperature RPMI 1640/B27 and combine with the cell suspension drop-wise with gentle mixing.
7. Slowly (over 2 minutes) add 8 mL of RPMI 1640/B27 to a 50 mL centrifuge tube.
8. Centrifuge at 300 g for 5 minutes at 20°C.
9. Carefully, using a 10 mL stripette remove 8 mLs of the supernatant. Remove a further 1 mL with a 1,000 µL pipette taking care not to disturb the cell pellet. Re-suspend the cells in the residual liquid (approx. 1 mL) with gentle agitation.

7.7. Determining Post-thaw Cell Viability of Cytiva Plus Cardiomyocytes for MPC (Time 30 minutes)

Determine the viable cell number and viable cell density using preferred method of choice. We use a NucleoCounter NC-100 (ChemoMetec) cell counter, the method for which is outlined below. However, this protocol could be adapted for other commercial cell counters.

Consumables

- RPMI 1640/B27 medium (filter sterilized)
- 4×1.5 mL tubes
- 4×NucleoCassettes™ (ChemoMetec)
- Reagent A100 (ChemoMetec)
- Reagent B (ChemoMetec)

Equipment

- 100 µL pipette & sterile 1-200 µL tips
- 1,000 µL pipette & sterile 1-1,000 µL tips
- NucleoCounter NC-100 (ChemoMetec)

Protocol

Perform the following step aseptically inside the biosafety cabinet (BSC).

1. Determine viable cell number using the NucleoCounter NC-100 (ChemoMetec). To do this, transfer 40 µL of the cell suspension into a 1.5 mL tube. Add to the tube 360 µL of RPMI 1640/B27 medium (i.e. now a 1:10 dilution). Gently agitate to achieve an even cell suspension.
2. The remaining steps can be performed outside of the BSC.
3. Transfer 100 µL of 1:10 diluted cell suspension into each of three more 1.5 mL tubes.
4. Use a 100 µL of 1:10 diluted cell suspension sample to calculate the number of Non-Viable Cells/mL (i.e. directly load the sample into a NucleoCassette).
5. Repeat step 4 for a second time, and calculate the average result.
6. Use another 1×100 µL of 1:10 diluted cell suspension sample to calculate the Total Number of Cells/mL. To do this, add 100 µL of Reagent A100, and then 100 µL of Reagent B, to the 100 µL of 1:10 diluted cell suspension sample. Mix by pipetting, then load into a NucleoCassette.
7. Repeat step 6 for a second time, and calculate the average result.
8. Determine the total Volume of Cell Suspension.

Note: We use a 1,000 µL pipette to establish this volume.

9. Calculate the number of viable cells in the cell suspension:

- Viable cells/mL = 10 × [(3 × Total Number of Cells/mL)-(Non-Viable Cells/mL)]
- Total number of viable cells = Viable cells/mL × Volume of Cell Suspension (mL)

Worked example for a typical 1.0×10^6 vial of Cytiva Plus Cardiomyocytes:

Total Number of Cells/mL determined in step 6 = 5.33×10^4

Non-Viable Cells/mL determined in step 5 = 4.80×10^4

Viable cells/mL = 10 × [(3 × 5.33×10^4) - (4.80×10^4)] = 1.12×10^6 cells/mL

Total number of viable cells = 1.12×10^6 cells/mL × 1 mL = 1.12×10^6 cells

Note: There are a total of 1.6×10^6 cells in a 1.0×10^6 vial of Cytiva Plus Cardiomyocytes. We routinely record a value of >70% post thaw cell viability (i.e. 1.12×10^6 viable cells).

7.8. Seeding Cytiva Plus Cardiomyocytes onto coverslips (Time 30 minutes)

Cells should be seeded at a density of 4.6×10^4 viable cells in 650 µL RPMI 1640/B27 per well of a 24-well cell culture plate (i.e. 7.1×10^4 viable cells/mL).

Note: Assuming the volume of the cell suspension is ~1 mL, and the number of viable cells is 1.12×10^6, the concentration is ~1.12×10^6 viable cells/mL. Consequently, the next step involves diluting the cell suspension to achieve the appropriate cell concentration.

Consumables

- RPMI 1640/ medium (filter sterilized)
- 60 mL bottle

Equipment

- 24-well culture plate containing Matrigel-coated coverslips
- 200 uL pipette & sterile 1-200 uL tips
- 37° water bath

Protocol

Perform the following steps aseptically inside the biosafety cabinet (BSC).

1. Warm 25 mL RPMI 1640/B27 medium in a sterile 60 mL bottle (sealed) in a 37°C water bath.
2. Dilute the cell suspension to 7.1×10^4 viable cells/mL using warm RPMI 1640/B27 medium.

$$\text{Required volume (mL)} = \frac{\text{Viable cells}}{7.1\times10^{4}\ \text{viable cells/mL}}$$

Example: A total viable count of 1.12×10^{6} cells would be divided by 7.1×10^{4} viable cells/mL to obtain a final required volume of 15.8 mL.

3. Aspirate the Matrigel from each well of the 24-well cell culture plate and **immediately** replace with 650 µL of Cytiva Plus Cardiomyocyte suspension (i.e. 7.1×10^{4} viable cells/mL).
4. After seeding all the required wells, put the lid on the cell culture plate and incubate in a standard cell culture incubator at 37°C, 5% CO_2.

7.9. Media change on day 4 post-thaw (Time 20 minutes)

1. Warm 30 mL RPMI 1640/B27 medium in a 60 mL bottle (sealed) in a 37°C water bath.
2. Perform the following steps aseptically inside the biosafety cabinet (BSC).
3. After 96 hours, carefully aspirate off 300 µL of the medium from each well, leaving 350 µL residual medium and replace with 300 µL of warmed RPMI 1640/B27 medium so as not to disturb the seeded cells. Incubate plate at 37°C, 5% CO2.
4. Repeat a media change at day 7 replacing half the seeded medium with fresh pre-warmed RPMI 1640/B27 medium.
5. Perform manual patch clamp 4–7 days after seeding in cell culture plates. It is the responsibility of the user to determine the optimal culture time for this application.

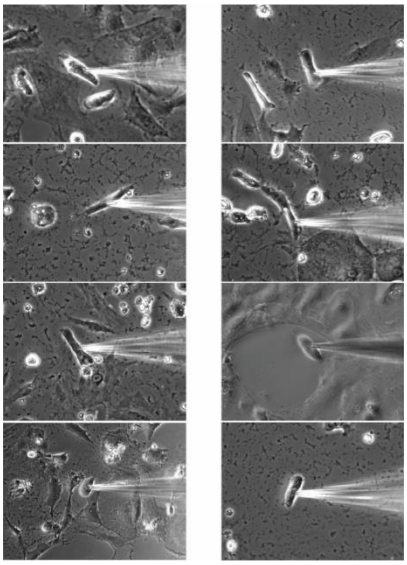


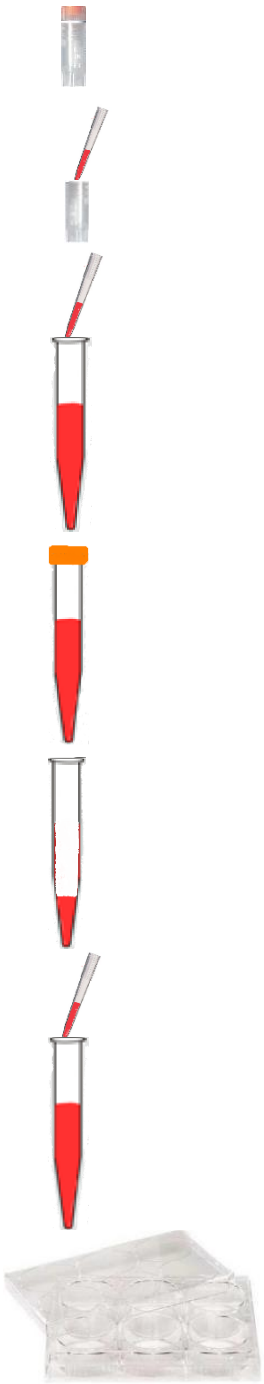
Figure 5 – Examples of cell morphologies patched manually.

8. Troubleshooting

Problems	Solutions
Poor cardiomyocyte viability	Check storage/shipping conditions. Follow pack leaflet to revive cells from cryostore. Follow recommended procedure for thawing and dilution of cardiomyocytes as improper handling of Cytiva Plus Cardiomyocytes could cause low viability. Ensure pre-warmed medium is added drop-wise to the cardiomyocytes.
Poor cell attachment	Make sure plates are tissue culture treated and sterile. Follow recommended procedure for preparation and storage of fibronectin plates. Aspirate fibronection just before seeding. Be careful not to touch the bottom of plate. Make sure the surface of the well has not been marked during the removal of fibronectin. Do not let the plate dry out. Place plate into incubator on a flat surface for 2 hours. Make sure viable count number is used to seed. Follow feeding steps as outlined. Ensure RPMI 1640/B27 is: a) warmed before use; b) used within 7 days of preparation; c) not exposed to light for extended periods; d) used within 7 days of preparation, and; e) not warmed repeatedly. Ensure that Matrigel coverage is uniform by microscopic inspection. Matrigel coated plates should be warmed to room temperature for at least 30 minutes before use.

Problems	Solutions
Uneven coating of Matrigel	Ensure culture vessels are stored on a level surface. Use only cell culture treated plastic ware.
Aggregation of Matrigel	Follow the recommended procedure for the preparation of Matrigel coating of plates. Avoid warming of Matrigel. Keep Matrigel on ice during processing if necessary. Avoid room temperature plastic coming in contact with Matrigel.
Poor ion channel activity	Allow at least 72-hour post thaw before use depending on the application. Ion channel activity will increase as cells recover from thaw. Ensure correct seeding density of Cytiva Plus for the application.
Poor performance on MEA	Do not allow the droplets of cell suspension (5.5.9) to dry out. The cell droplet should still be visible post 2–3 hour incubation prior to adding medium to the well (5.5.10). Take care when adding the medium to the well so as not to disturb the seeded cells (5.5.10). If using re-usable MEA plates, ensure that the MEA plates are: (A) Sterile and the plating surface is dry and free of cellular debris. (B) Maintained and stored as directed by the manufacturer.

9. Product Protocol Flowchart



Thaw vial of cardiomyocytes at 37° C. Remove vial contents to a 50 mL tube with a 1 mL pipette.

↓

Rinse vial with 1 mL RPMI 1640/B27 medium and slowly add to the cardiomyocytes.

↓

Add a further 8 mLs of RPMI 1640/B27 medium to the cell suspension.

↓

Centrifuge cell suspension at 300 g for 5 minutes.

↓

Remove supernatant and re-suspend cell pellet in RPMI 1640/B27 medium. Perform a cell count.

↓

Dilute cell suspension to required cell density.

↓

Dispense into appropriate cell culture device. Incubate at 37°C for 4-7 days depending on application. Feed 4 days post thaw and every 3 days thereafter with RPMI 1640/B27 medium.