

User Guide of **CardioEasy**[®] Human Cardiomyocytes on the Axion Maestro MEA



Contents

1.	Introduction ······ 3
2.	Consumables and Equipment 4
3.	Experimental Workflow4
4.	Experimental Procedures4
5.	Representative Data5

1. Introduction •

CardioEasy[®] Human Cardiomyocytes constitute a ready-to-use, high-purity cardiac cell product. These cells are manufactured through Cellapy's proprietary efficient cardiac differentiation and purification technology. The entire processes including culture and differentiation of human pluripotent stem cells (hiPSCs), utilize chemically-defined media components.

The CardioEasy[®] Human Cardiomyocytes are primarily composed of ventricular-like cardiomyocytes exhibiting spontaneous electrophysiological activity, along with minor subpopulations of atrial-like and sinoatrial node-like cells. These cells express canonical cardiac-specific genes, including various contractile proteins and ion channels. Functionally, they demonstrate classic cardiomyocytes electrophysiological properties and exhibit physiologically typical responses to both electrophysiological and biochemical stimuli.

As evidenced by electrophysiological and calcium transient signal recordings, these cells serve as an optimized in vitro model for:

- New drug screening.
- Cardiotoxicity assessment.
- Disease mechanism research.

Microelectrode Array (MEA) is a technique for synchronized recording of cellular or tissue electrical activity via high-density microelectrodes. These systems typically integrate dozens to thousands of microelectrodes, enabling network-level electrophysiological monitoring of excitable cells (e.g., neurons, cardiomyocytes) with high-throughput capability and superior spatiotemporal resolution.

Primary applications include:

- Cardiotoxicity assessment (hERG channel screening).
- Neuroscience research (brain-computer interfaces, neural network decoding).
- Organ-on-chip functional analysis.

Modern MEA systems integrate optical imaging and microfluidic technologies, enabling multimodal detection and providing critical technical support for understanding cellular electrophysiological mechanisms and disease modeling. More important, this technology enables high-content cardiotoxicity screening at industrial scale.



2. Consumables and Equipment •

Consumables and Equipment	Company	Catalog Number
Human Cardiomyocytes Coating Solution	Cellapy	CA2010008
Human Cardiomyocytes Recovery & Seeding Medium	Cellapy	CA2002008
Human Cardiomyocytes Maintenance Medium	Cellapy	CA2015002
Human Cardiomyocytes - Drug Screening Grade	Cellapy	CA2201106
CytoView MEA 24 CytoView-Z 96	Axion Biosystems	M384-tMEA-24W Z96-IMP-96B
Maestro Edge Maestro Z	Axion Biosystems	MAESTRO384EDGE MAESTRO96Z

3. Experimental Workflow •

Coating electrode plates \rightarrow Thawing and seeding cardiomyocytes \rightarrow Medium change and culture \rightarrow On-machine detection

4. Experimental Procedures •

- 4.1 Coat the electrode plates with cardiomyocytes coating solution (5 μL/well) and incubate overnight at 37°C. Add sterile water to the inter-well and inter-plate gaps.
- 4.2 Thaw cells at 37°C with gentle agitation until only a small ice crystal remains (avoid submerging the tube cap). Quickly retrieve the vial, disinfect the surface with 75% ethanol, and transfer it to a biosafety cabinet.
- 4.3 Carefully transfer the cell suspension to a fresh 15 mL centrifuge tube using a 1 mL pipette, avoiding vigorous pipetting.

- 4.4 Rinse the cryovial with 1 mL of human cardiomyocytes maintenance medium to collect residual cells. Gradually add the rinse solution to the cell suspension tube at a rate of 1 drop/5 seconds while gently swirling the tube. Repeat this process by adding 4 mL of maintenance medium dropwise with continuous mixing.
- ► 4.5 Centrifuge at 200 g for 5 mins, discard the supernatant, and resuspend in an appropriate volume of cardiomyocytes recovery/seeding medium. Perform trypan blue staining and cell counting, then adjust the cell density to 2×10⁴ cells/well. For a 24-well electrode plate, prepare 5×10⁵ cells in 125 µL total suspension volume.
- ► 4.6 Seed cardiomyocytes onto electrode plates: Before seeding each well, aspirate the pre-coated solution. Mix the cell suspension thoroughly and carefully pipette 5 µL to the center of each well without touching the bottom.
- ► 4.7 After seeding, incubate the plate in a CO₂ incubator. After 8 h or overnight, observe cell attachment microscopically. Tilt the plate at 45°, add 300 µL fresh cardiomyocytes maintenance medium to each well, and continue culture with medium changes every 3 days.
- 4.8 Electrophysiological recording: Set Maestro Edge parameters to 37°C and 5% CO₂, using CardioEasy[®] Human Cardiomyocytes Maintenance Medium. Refresh medium one day prior to recording. Initiate data acquisition after 10 mins equilibration. Typically, weak electrical signals emerge at day 2-3 post-thaw, reaching normal intensity by day 5-7, at which point cells are ready for subsequent experiments.

5. Representative Data •

► 5.1 Morphological Images of CardioEasy[®] Human Cardiomyocytes Cultured on CytoView MEA 24-Well White Plate.



Figure 1. Morphological images of CardioEasy[®] Human Cardiomyocytes transfected with GFP lentivirus and cultured on CytoView MEA[™] 24-well white plate.



▶ 5.2 Consistency of CardioEasy[®] Human Cardiomyocytes Baseline Data.

Baseline Field Potential recordings of CardioEasy[®] Human Cardiomyocytes cultured on CytoView MEA 24-well white plate were measured on Day 7 post-seeding.



Figure 2. Spike Amplitude data from CytoView MEA 24-well white plate (amplitude threshold set at 500 μ V). Valid signals were detected in 375/384 electrodes (97.7%).



Figure 3.Beat Rate data from CytoView MEA 24-well white plate. Inter-well beat intervals were maintained between 1300-1600 ms.





Figure 4. After 10 minutes of instrument monitoring, the Beat Period of cardiomyocytes contraction (as shown in the figure) indicates stable cellular beating, confirming suitability for subsequent drug administration experiments.



Figure 5. Representative real-time field potential traces from 16 electrodes within the well following cardiomyocytes electrical signal stabilization.

	1	2	3	4	5	6		
1	300	365	370	315	368	351		
2	399	303	287	321	376	375	average	349
3	369	310	316	366	338	381	std dev	35.1
4	302	336	363	371	420	371	%cv	10.06

FPDc (Fridericia ms)

Table 1. Representative Field Potential Duration (Corrected, FPDc) data sets per well for cardiomyocytes.

Spike Amplitude (mV)

	1	2	3	4	5	6		
1	10.2	11.1	6.3	9.8	8.2	6.3		
2	6.6	9.9	7.7	9.3	8.4	6.3	average	9
3	9.6	6.6	6.6	5.1	8.6	8.5	std dev	2.0
4	12.5	8.9	8.0	13.0	9.5	9.6	%cv	23.15

Table 2. Representative Spike Amplitude data per well for cardiomyocytes.

Beat Period (S)									
	1	2	3	4	5	6			
1	1.4	1.4	1.4	1.4	1.5	1.6			
2	1.4	1.3	1.4	1.3	1.3	1.4	average	1.4	
3	1.4	1.4	1.4	1.4	1.4	1.4	std dev	0.1	
4	1.5	1.5	1.4	1.4	1.3	1.4	%cv	4.09	

Table 3. Representative Beat Period Data per well for cardiomyocytes.



► 5.3 Acute Cardiotoxicity Data.

Figure 6. CardioEasy[®] Human Cardiomyocytes cultured on CytoView MEA 24-white plate for 7 days underwent baseline Field Potential recording, followed by pharmacological intervention. The bar chart depicts concentration-dependent FPDc



prolongation post-treatment with various compounds (Dofetilide, Quinidine, Sotalol, and Vandetanib).



5.4 Chronic Cardiotoxicity Data.

Figure 7. CardioEasy[®] Human Cardiomyocytes was analyzed using the MAESTRO Z system, with doxorubicin (Dox) treatment administered 142 hours post-seeding. Cells were treated with 0.5 μ M (Dox-1), 1 μ M (Dox-2), and 2 μ M (Dox-3) doxorubicin, followed by real-time monitoring. Panel A displays the cellular impedance changes throughout the experimental period. The graph shows the KT50 values (time required to kill 50% of cells) for each doxorubicin treatment group. Results demonstrate concentration-dependent cardiotoxicity, where higher doxorubicin concentrations produced stronger cytotoxic effects, as evidenced by progressively lower KT50 values.