

Measuring Cardiac Activity: *Impedance and Extracellular Field Potential Detection with xCELLigence RTCA CardioECR System*

Introduction

iCell® Cardiomyocytes², human cardiomyocytes derived from induced pluripotent stem cells, have been optimized for rapid recovery from cryopreservation. As an extension of the validated iCell Cardiomyocytes product line, they fully recapitulate biochemical, electrophysiological, mechanical, and pathophysiological characteristics of native human cardiac myocytes. These properties combine to make iCell Cardiomyocytes² an optimal in vitro test system for interrogating cardiac biology in basic research and many areas of drug development.

The xCELLigence RTCA CardioECR System (RTCA CardioECR system) is a non-invasive, label-free platform that combines field potential and impedance recording for simultaneous measurement of electrical and contractile activity, respectively. This platform allows comprehensive evaluation of viability, contractility, and electrical activity involved in excitation-contraction (EC) coupling across the cardiomyocyte monolayer. iCell Cardiomyocytes² can be cultured and maintained in an E-Plate for extended durations, thus enabling measurement of acute and sub-acute drug-induced effects. Together, iCell Cardiomyocytes² and the RTCA CardioECR system offer an excellent platform for in vitro screening of compound effects on human cardiomyocyte physiology.

This Application Protocol describes how to handle iCell Cardiomyocytes² for use on the RTCA CardioECR system and provides basic instructions for compound treatments, data acquisition, and analysis.

Required Equipment, Consumables, and Software

The following equipment, consumables, and software are required in addition to the materials specified in the iCell Cardiomyocytes² User's Guide.

Item	Vendor	Catalog Number
Equipment		
8-channel Multichannel Pipettor, 20 and 200 µl	Multiple Vendors	
xCELLigence RTCA CardioECR System	ACEA Biosciences	
Consumables		
iCell Cardiomyocytes ² Kit (Cardiomyocytes)	Cellular Dynamics International (CDI)	CMC-100-012-000.5 (0.5 unit) CMC-100-012-001 (1 unit)
Dulbecco's Phosphate Buffered Saline without Ca ²⁺ and Mg ²⁺ (D-PBS)	Invitrogen	14190
E-Plate Cardio 48 (E-Plate)	ACEA Biosciences	0030060094000300600950
Fibronectin	Roche Applied Science	11051407001 11080938001

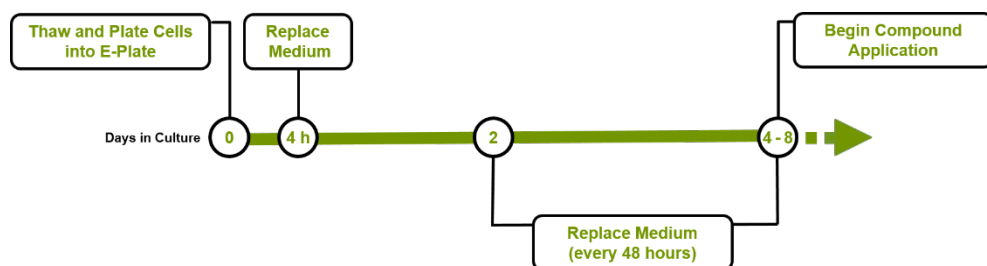
Item	Vendor	Catalog Number
Sterile Reagent Reservoirs	Multiple Vendors	
Software		
RTCA CardioECR Instrument Software	ACEA Biosciences	

Notes

Workflow

The cardiomyocytes are thawed and plated into an E-Plate previously coated with fibronectin. 4 hours post-plating and every 48 hours thereafter, spent medium is replaced with iCell Cardiomyocytes Maintenance Medium (Maintenance Medium). From day 4 - 8 post-plating, cells can be treated with compounds, and the cardiac activity recorded.

Note: An alternative weekend-free workflow may be acceptable. Contact CDI's Technical Support (support@cellulardynamics.com; +1 (877) 320-6688 (US toll-free) or (608) 310-5100) for more information.



Methods

Preparing the E-Plate

The E-Plate is prepared the day of plating cardiomyocytes.

1. Dilute 1 mg/ml fibronectin solution in sterile D-PBS to a final concentration of 10 µg/ml immediately before use.

Note: Reconstitute fibronectin in sterile water at 1 mg/ml according to the manufacturer's instructions. Aliquot and store at -20°C.

2. Add 50 µl/well of the 10 µg/ml fibronectin solution to the center of the wells of an E-Plate to evenly coat the bottom of the well.
3. Incubate at 37°C for at least 1 hour.

Thawing Cardiomyocytes

1. Aspirate the fibronectin solution from the E-Plate. Immediately add 50 µl/well of 37°C iCell Cardiomyocytes Plating Medium (Plating Medium) to the center of the wells.

Note: Do not allow the fibronectin-coated surface to dry.

2. Equilibrate the E-Plate in a cell culture incubator at 37°C, 5% CO₂ for 5 - 10 minutes.
3. Record a background measurement according to the RTCA CardioECR Instrument Operator's Guide.
4. Thaw the cardiomyocytes according to their User's Guide to a final volume of 5 ml Plating Medium by diluting the 1 ml cell suspension from the cryovial in 1 ml of Plating Medium rinse and 3 ml of additional Plating Medium.
5. Remove a sample of cells to confirm viability using a hemocytometer (using trypan blue exclusion to identify viable cells) or an automated cell counter.
6. Calculate the final volume of Plating Medium needed to obtain a final cell plating density of 1 x 10⁶ viable cardiomyocytes/ml using the number of viable cells/vial from the Certificate of Testing.
7. Remove the E-Plate from the RTCA CardioECR Instrument and equilibrate to room temperature for 5 - 10 minutes.
8. Add 50 µl/well of the cell suspension (50,000 cells/well) to the center of the wells using a multichannel pipettor.
9. Leave the E-Plate undisturbed in the biological safety cabinet at room temperature for 20 - 30 minutes to allow the cardiomyocytes to settle and ensure an even distribution.
10. Culture the cardiomyocytes in a cell culture incubator at 37°C, 5% CO₂ for 4 hours.

Note: Place the E-Plate in a low traffic incubator and away from the door to minimize fluctuations in temperature and air movement.

Maintaining Cardiomyocytes in the E-Plate

1. Immediately before use, equilibrate an aliquot of Maintenance Medium in a 37°C water bath.
2. Replace the Plating Medium with Maintenance Medium 4 hours post-plating. Tilt the E-Plate, remove the spent medium using a multichannel pipettor, and gently add 100 µl/well of 37°C Maintenance Medium to the side of the well to avoid disturbing the cardiomyocyte monolayer.

Note: Do not allow the pipettor tips to touch the bottom of the well during medium removal or addition. Medium replacement may cause transient alterations to beating rhythm. Allow normal beating patterns to recover after medium replacement prior to drug application.

3. Maintain the cardiomyocytes on the E-Plate replacing 100% of the spent medium with Maintenance Medium every 48 hours.
4. Culture the cardiomyocytes in a cell culture incubator at 37°C, 5% CO₂.
5. Perform recordings from day 4 - 8 post plating.

Data Acquisition and Analysis

The RTCA CardioECR Software (RTCA software) offers a wide variety of options for data acquisition and analysis. The instructions here are meant to provide a general guidance. See the RTCA CardioECR Instrument Operator's Guide for specific instructions.

The beating pattern stabilizes on day 4 post-plating cardiomyocytes into the E-Plate, when the cells can be treated with compounds and the assay can be performed to analyze the electrical activity. Example baseline activity can be seen in Figure 1.

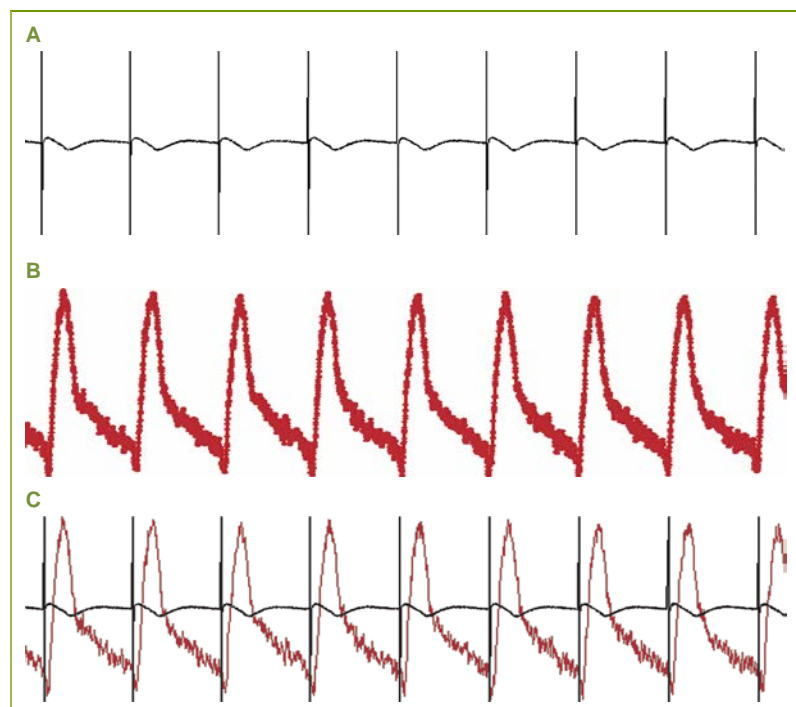


Figure 1: iCell Cardiomyocytes² and the RTCA CardioECR System Enable Multiplexed Endpoints for Easy and Robust Interrogation of Cardiomyocyte Function

Electrical and contractile activity define the excitation-contraction process. The example traces show simultaneous 10 second recordings where (A) electrical activity is recorded as the extracellular field potential and (B) contractile activity is captured as changes in impedance readings from iCell Cardiomyocytes². (C) The overlay of these recordings illustrate how the processes are linked and the ease with which compounds can be assessed for effects on electrical, contractile, or both activity.

Applying Compounds

1. Immediately before use, equilibrate an aliquot of Maintenance Medium in a 37°C water bath.
2. Replace the Maintenance Medium 4 - 24 hours before recording. Tilt the E-Plate, remove the Maintenance Medium using a multichannel pipettor, and gently add 90 µl/well of Maintenance Medium to the side of the well to avoid disturbing the cardiomyocyte monolayer.

Note: *Evaporation rates can vary across the E-Plate. Changing the Maintenance Medium before compound treatment is required to ensure uniform medium volumes across the E-Plate.*

Notes

Notes

3. Culture the cardiomyocytes in a cell culture incubator at 37°C, 5% CO₂.
4. Monitor the activity of the cardiomyocytes on the E-Plate to ensure regular beating rate and stable whole-peak amplitude values are reached.
5. Prepare test compounds in Maintenance Medium at 10X the final concentration in a regular 48-well cell culture plate.

Note: Final DMSO concentrations above 0.1% should be used with caution. Therefore, if test compounds are dissolved in DMSO, the 10X compound solutions should not exceed 1% DMSO.

6. Equilibrate the 48-well cell culture plate containing the 10X compound solutions in a cell culture incubator at 37°C, 5% CO₂.
7. Quickly transfer 10 µl/well of the 10X compound solutions from the 48-well cell culture plate to the E-Plate. Gently mix by pipetting 3 - 5 times.

Note: Beating rate and amplitude are temperature-dependent. The E-Plate should not be kept outside the incubator for more than 5 minutes while compounds are added.

Data Acquisition and Analysis Using the RTCA Software

See the RTCA CardioECR Instrument Software Guide for specific instructions on using the RTCA software for data acquisition and analysis.

Example Data

Beating rate, amplitude, and beating rhythm irregularity were calculated with the RTCA software. Data were normalized to the last measurement point before compound treatment and averaged across the replicate wells. Results displayed in Figures 2 and 3 were generated with recordings acquired 30 minutes after drug treatment compared to untreated wells.

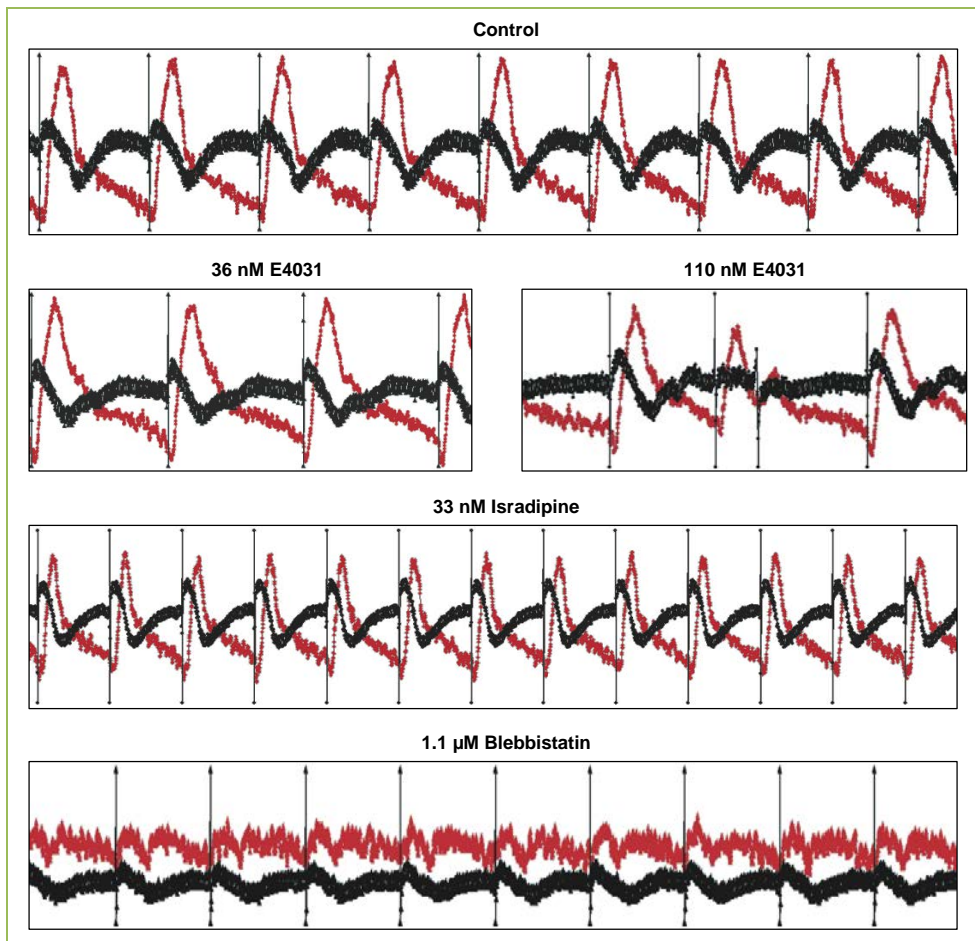


Figure 2: iCell Cardiomyocytes² and the RTCA CardioECR System Enable Simultaneous Detection of Different EC Endpoints

Modulating ion channel and mechanical cardiac activity alters the spontaneous electrical and contractile activity of iCell Cardiomyocytes². Blocking I_{Kr} and I_{Ca-L} with E4031 and isradipine, respectively, produced the expected phenotypic effects on both field potential and impedance waveforms. Blocking mechanical activity with the myosin II ATPase inhibitor blebbistatin resulted in the abrogation of the impedance signal without affecting the electrical activity as indicated by the unaltered field potential signal.

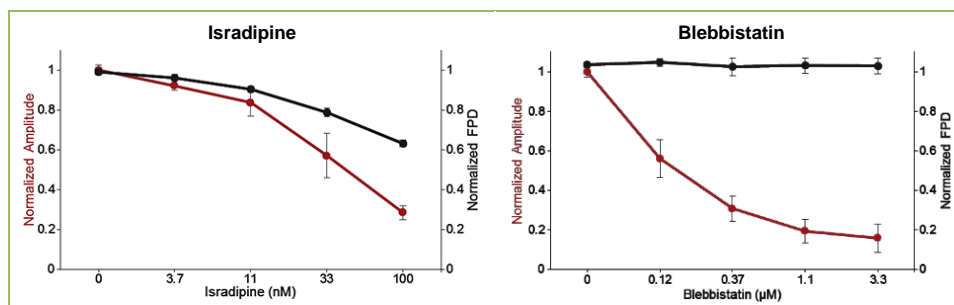



Figure 3: Class-specific Phenotypic Responses Are Easily Characterized and Quantified in iCell Cardiomyocytes² on the RTCA CardioECR System

Blocking I_{Ca-L} and the myosin II ATPase with isradipine and blebbistatin exemplifies how differences between electrical (channel block) and mechanical (contraction antagonists) drug-induced effects can be detected and separated through analysis of impedance (red) and field potential (black) signals (mean \pm SEM; $n = 3$ wells for each condition).

Summary

iCell Cardiomyocytes² provide an in vitro test system that equilibrates rapidly upon reanimation from cryopreservation to recapitulate native human cardiac myocyte physiology and function while the RTCA CardioECR system provides a label-free technology for non-invasive monitoring of electrical and mechanical cellular functions. The methods and results presented here highlight the ease of use with which robust and relevant data can be gathered on human cardiomyocyte viability, electrical activity, and contractility. This system enables quick detection and discrimination of compound effects on different components of the EC coupling. Together these tools bring 48-well based, real-time, predictive assessments of compound efficacy, potency, and toxicity on human cardiomyocytes to the drug development process.

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