Measuring Cardiac Activity:
Intracellular Calcium Flux Detection with FDSS/µCELL

Introduction

iCell® Cardiomyocytes² are human cardiomyocytes that recapitulate the electrophysiological, biochemical, mechanical, and pathophysiological characteristics of native human cardiac myocytes. Their derivation from human induced pluripotent stem (iPS) cells, high purity, functional relevance, and ease of use, make iCell Cardiomyocytes² an optimal in vitro test system for interrogating cardiac biology in basic research and many areas of drug development.

The FDSS/µCELL Functional Drug Screening System (FDSS/µCELL) is a kinetic plate-based, cellular assay screening system that utilizes calcium fluorescent dyes to measure calcium flux and detect cardiomyocyte beating activity. iCell Cardiomyocytes² can be cultured on 96- or 384-well plates where they form a stable, electrically and mechanically active syncytium amenable to measuring drug-induced perturbations. Together, iCell Cardiomyocytes² and the FDSS/µCELL offer a high-throughput platform for in vitro screening of compound efficacy and toxicity in human cardiac myocytes.

This Application Protocol describes how to handle iCell Cardiomyocytes² for use on the FDSS/µCELL and provides basic instructions for compound treatment, 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.

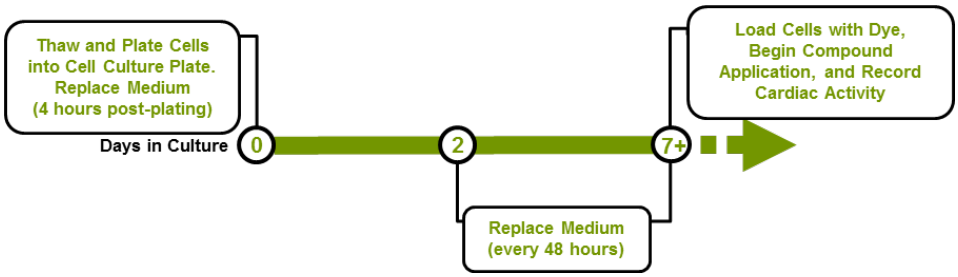
Item	Vendor	Catalog Number
Equipment		
FDSS/µCELL Functional Drug Screening System (FDSS/µCELL)	Hamamatsu	
Consumables		
iCell Cardiomyocytes² Kit	Cellular Dynamics International (CDI)	CMC-100-012-000.5 CMC-100-012-001
EarlyTox Cardiotoxicity Kit	Molecular Devices	R8210
FDSS/µCELL Pipette Tips, Black, Non-sterile (Pipette Tips)*	Hamamatsu	A8787-32 – 96-well format A8687-62 – 384-well format
Flat Clear Bottom Black Polystyrene TC-treated Microplate (Cell Culture Plate)*	Multiple Vendors	
Software		
Data Analysis Unit Package	Hamamatsu	

* Order the format compatible with the pipette head of the FDSS/µCELL.

Workflow

iCell Cardiomyocytes² are thawed and plated into either a 96- or 384-well cell culture plate previously coated with gelatin. 4 hours post-plating and every 48 hours thereafter, spent medium is replaced with iCell Cardiomyocytes Maintenance Medium (Maintenance Medium). From day 7 post-plating, cells can be loaded with calcium fluorescent dye, treated with compounds, and assayed for calcium flux and beating activity.

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

The following procedure describes culturing iCell Cardiomyocytes² in 96-well cell culture plates. Instructions for 384-well format are provided in the notes.

Thawing iCell Cardiomyocytes²

1. Coat a 96-well cell culture plate with 100 µl/well of 0.1% gelatin solution at 37°C for at least 1 hour according to the iCell Cardiomyocytes² User's Guide.

   Note: *For 384-well format, coat with 33 µl/well of 0.1% gelatin solution*.

2. Calculate the final volume of iCell Cardiomyocytes Plating Medium (Plating Medium) needed to obtain a final cell plating density of 0.5 x 10^6 viable cardiomyocytes/ml using the number of viable cells/vial from the Certificate of Testing.

3. Thaw iCell Cardiomyocytes² according to the User's Guide to the calculated final volume of Plating Medium.

4. Aspirate the gelatin solution. Immediately add 100 µl/well of the iCell Cardiomyocytes² cell suspension (50,000 cells/well).

   Note: *For 384-well format use the same iCell Cardiomyocytes² cell suspension density (500,000 viable cells/ml) and add 33 µl/well (~16,500 cells/well).*

5. Culture iCell Cardiomyocytes² in a cell culture incubator at 37°C, 5% CO_2 for 4 hours.

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Maintaining iCell Cardiomyocytes²

1. Aspirate the spent Plating Medium 4 hours post-plating and replace with 100 µl/well of Maintenance Medium.

   Note: *For 384-well format, replace with 33 µl/well of Maintenance Medium.*

2. Maintain the cardiomyocytes for at least 7 days, replacing spent Maintenance Medium every 48 hours.

Data Acquisition and Analysis

The following section details loading iCell Cardiomyocytes² with a Ca^{2+}-sensitive dye, data acquisition, and basic data analysis. Be aware that iCell Cardiomyocytes² beat rate is highly temperature-sensitive. Avoid temperature fluctuations by maintaining the cell culture plate containing iCell Cardiomyocytes² and the plate containing test compounds dilutions (compound plate) at 37°C throughout the assay.

FDSS analysis software provides a variety of parameters to evaluate the calcium oscillations of iCell Cardiomyocytes². See the manufacturer's instructions for specific guidelines on using FDSS analysis software for data acquisition and analysis.

Note: *The FDSS analysis software is provided in the Data Analysis Unit Package.*

Adding the Dye and Applying Compounds

1. Prepare the Cardiotox Dye loading solution according to the manufacturer's instructions.

   Note: *The Cardiotox Dye is provided in the EarlyTox Cardiotoxicity Kit.*

2. Prepare a 1:1 mixture of freshly prepared Cardiotox Dye and Maintenance Medium.

3. Remove the 96-well cell culture plate containing iCell Cardiomyocytes² from the cell culture incubator and replace the spent medium with 90 µl/well of the Cardiotox Dye and Maintenance Medium mixture.

   Note: *For 384-well format, replace spent medium with 40 µl/well of Cardiotox Dye and Maintenance Medium mixture.*

4. Incubate the cell culture plate containing iCell Cardiomyocytes² and Cardiotox Dye loading solution (assay plate) in a cell culture incubator at 37°C, 5% CO_2 for 2 hours. Do not remove the plate from the incubator until ready to transfer to the FDSS/µCELL.

5. Equilibrate the FDSS/µCELL to 37°C.

6. Prepare test compound dilutions in Maintenance Medium at 10X the final concentration in a sterile 96-well plate (compound plate). Incubate the compound plate at 37°C until ready to apply compounds onto iCell Cardiomyocytes².

   Note: *For 384-well format, prepare compound dilutions in Maintenance Medium at 5X the final concentration.*

   Note: *Final DMSO concentrations above 0.2% should be used with caution. If test compounds are dissolved in DMSO, the 5X compound solutions should not exceed 0.5% DMSO.*

7. After incubation, place a new box of pipette tips onto the FDSS/µCELL and immediately transfer the assay plate and the compound plate from the cell culture incubator directly to the FDSS/µCELL equilibrated to 37°C.

   Note: *Do not rinse the cells after adding the Cardiotox Dye loading solution.*

8. Record a baseline measurement (60 seconds) according to the FDSS/µCELL and the EarlyTox Cardiotoxicity Kit guides.

   Note: *See the FDSS/µCELL or EarlyTox Cardiotoxicity Kit guide for specific instrument settings and recommendations on how to adjust parameters to optimize the cellular response.*

9. Transfer 10 µl/well of the 10X compound dilutions from the compound plate to the assay plate, maintaining both plates on the FDSS/µCELL equilibrated to 37°C. Record a simultaneous compound treatment measurement to detect immediate/short-term responses.

   Note: *For 384-well format, transfer 10 µl/well of the 5X compound dilutions.*

10. After the compound treatment recording, return the assay plate to the cell culture incubator. Intermediate and long-term responses can be detected with subsequent measurements (i.e. 5, 15, 30, and 60 minutes post-compound treatment).

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Example Data

Results displayed in Figures 1 and 2 were obtained 15 minutes post-compound treatment. These figures illustrate representative calcium oscillation waveforms and the effects of modulating crucial ion channel and GPCR through compound addition.

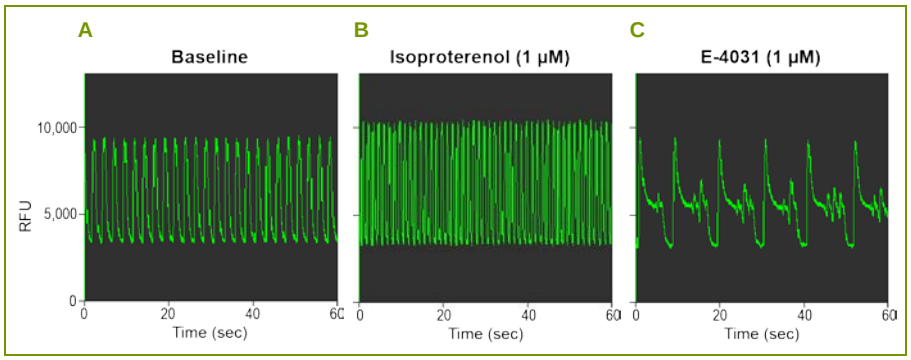

Figure 1: Intracellular Ca^{2+} Oscillations Provide a High-throughput Readout for Ion Channel and GPCR Function

Electrical activity at the membrane is controlled by ion channels and GPCRs. This activity regulates intracellular Ca^{2+} oscillations, which can thus be used as a high-throughput measure of cardiac excitation-contraction coupling. Panel A shows calcium oscillation waveforms under control conditions (baseline). Panels B and C show the effect of the GPCR-agonist isoproterenol and the I_{Kr} channel antagonist E-4031, respectively, on the calcium oscillation waveforms. iCell Cardiomyocytes² were exposed to the indicated compounds at the concentrations listed.

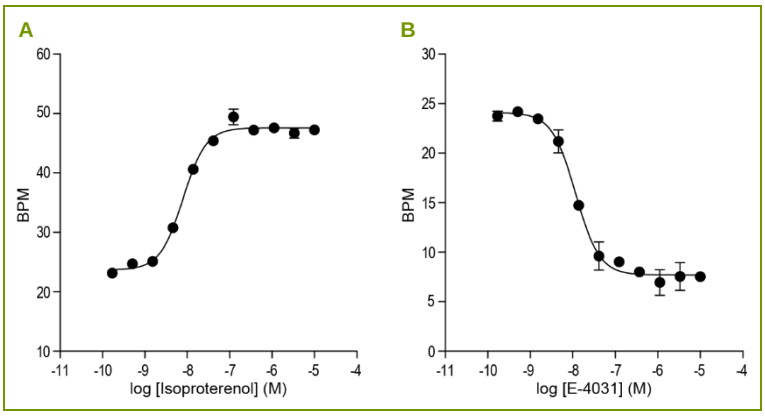

Figure 2: iCell Cardiomyocytes² Display Expected Response to Known Pharmacological Stimuli

Peak frequency (beats per minute, BPM) were calculated using the FDSS analysis software to analyze pharmacological modulation of iCell Cardiomyocytes² calcium oscillations after treatment with the GPCR-agonist isoproterenol or the I_{Kr} channel antagonist E-4031. Data are presented as raw values, mean ± SEM.

Summary

iCell Cardiomyocytes² provide an in vitro test system that recapitulates native human cardiac myocyte physiology and function while the FDSS/µCELL provides a high-throughput platform for measuring cardiomyocyte behavior. The methods and results presented here highlight the ease of use with which robust and relevant data can be generated on human cardiomyocyte contractile activity. Together, these tools bring 96- and 384-well based predictive assessments of compound efficacy and toxicity on human cardiomyocytes to the drug development process.

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