FUJIFILM Cellular Dynamics

iCell® Cardiomyocytes²

Application Protocol

nanion

Measuring Cardiac Contractility with FLEXcyte 96 System

Introduction

iCell® Cardiomyocytes², human cardiomyocytes derived from induced pluripotent stem cells, have been optimized for rapid recovery from cryopreservation. The iCell Cardiomyocytes product line fully recapitulates 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 areas of drug development and testing.

FLEXcyte 96, an add-on to the CardioExcyte 96 system, uses 96-well plates with flexible silicone membranes. This system enables contractility measurements of cells cultured in a more physiological environment, thus reflecting the mechanical conditions of the native human heart. iCell Cardiomyocytes² can be cultured and maintained in a FLEXcyte 96 plate for extended durations allowing for the measurement of acute and sub-acute drug-induced effects. Together, iCell Cardiomyocytes² and the FLEXcyte 96 system offer an excellent platform for *in vitro* screening of compound effects on human cardiac contractility. This Application Protocol describes how to handle iCell Cardiomyocytes² for use on the FLEXcyte 96 system and provides basic instructions for compound treatments, data acquisition, and analysis.

Required Equipment, Consumables, and Software

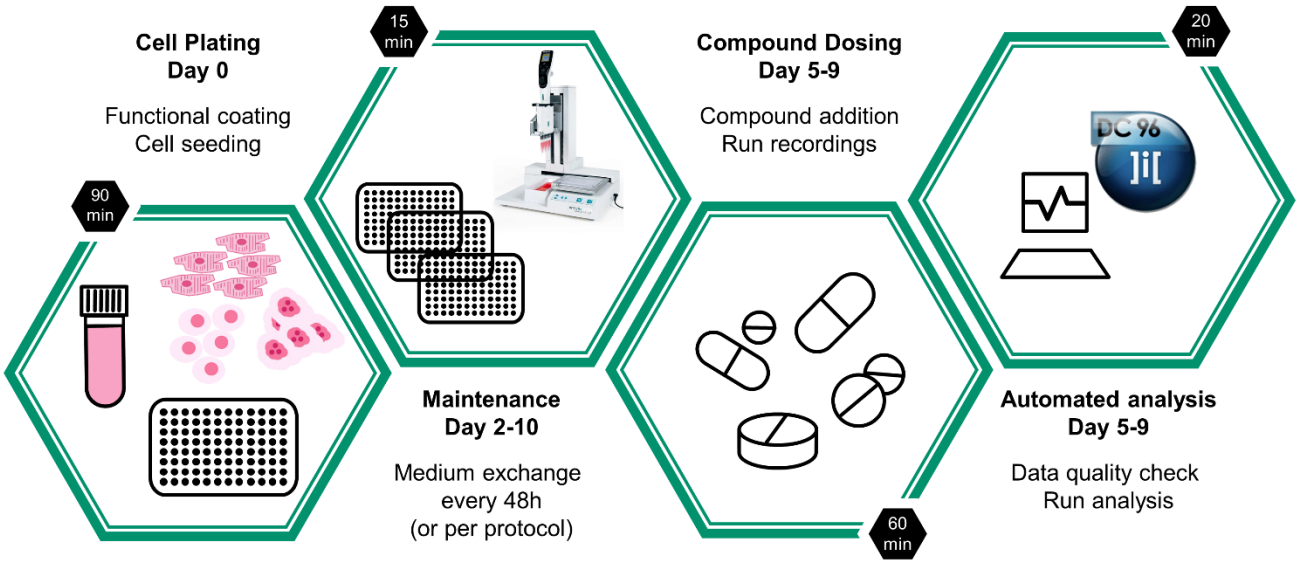
The following equipment, consumables, and software are required in addition to the materials specified in the iCell Cardiomyocytes² User's Guide.

Item	Vendor(s)	Catalog Numbers
Equipment		
Multichannel pipettor:8 or 12 channels	Multiple Vendors	
FLEXcyte 96	Nanion Technologies GmbH	
Consumables		
iCell Cardiomyocytes² Kit, 01434 or 11713	FUJIFILM Cellular Dynamics, Inc. (FCDI)	R1017, R1218 or R1219
• iCell Cardiomyocytes Plating Medium, 30 ml	FUJIFILM Cellular Dynamics, Inc. (FCDI)	(included in kit)
• iCell Cardiomyocytes Maintenance Medium, 100 ml	FUJIFILM Cellular Dynamics, Inc. (FCDI)	(included in kit)
DPBS, no calcium, no magnesium	Cytiva	SH30264.01
FLX-96 FLEXcyte Sensor Plates (FLX-96)	Nanion Technologies GmbH	201010
Fibronectin (Human Plasma)	FUJIFILM Wako Chemicals	063-05591
Sterile Reagent Reservoirs	Multiple Vendors	

Software	
CardioExcyte Control Software	Nanion Technologies GmbH
DataControl96 Software	Nanion Technologies GmbH

Workflow

The cardiomyocytes are thawed and plated into a FLX-96 plate previously coated with fibronectin using iCell Cardiomyocytes Plating Medium. After overnight cell adhesion, replace the iCell Cardiomyocytes Plating Medium with iCell Cardiomyocytes Maintenance Medium and every 48 hours thereafter. Starting day 5 post-plating, cells can be treated with test compounds and the cardiac contractility recorded. Resulting data acquired can be analyzed with DataControl96 Software.



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

Tips Before Starting

1. Refer to the iCell Cardiomyocytes[2] User's Guide for information on storage and handling of the cells and media.
2. Read this entire Application Protocol before handling iCell Cardiomyocytes[2] to become familiar with assay workflow.
3. Thaw both bottles of media required for this assay, including iCell Cardiomyocytes Plating Medium (30 ml) and iCell Cardiomyocytes Maintenance Medium (100 ml), overnight at 4°C the day prior to thawing and plating cells.

Methods

Coating the FLX-96 Plate with Fibronectin

The FLX-96 plate is prepared the day of plating the iCell Cardiomyocytes^2.

1. Prepare a 10 µg/ml working solution of fibronectin by diluting the 1 mg/ml stock solution 1:100 in sterile DPBS without Ca^{2+} and Mg^{2+} immediately before use.

   Note: *To prepare a fibronectin stock solution, reconstitute fibronectin in sterile water at 1 mg/ml according to the manufacturer's instructions. Aliquot and store at -20°C.*

2. Add 100 µl/well of the 10 µg/ml fibronectin solution to the center of the wells of a FLX-96 plate to evenly coat the bottom of the well.

3. Incubate the fibronectin-coated FLX-96 plate in a cell incubator at 37°C for at least 3 hours.

Thawing iCell Cardiomyocytes^2

1. Thaw the cells into a sterile 50 ml centrifuge tube according to the iCell Cardiomyocytes^2 User's Guide.

   Note: *The total volume of cell suspension at thaw is 5 ml (1 ml cryovial contents + 1 ml Plating Medium rinse + 3 ml of additional Plating Medium), which is less than the amount listed in Chapter 5 of the User's Guide.*

2. To confirm cell viability and count, remove a sample of cells to be examined in a hemocytometer with trypan blue or an automated cell counter.

3. Calculate the final volume of iCell Cardiomyocytes Plating Medium needed to obtain a final cell plating density of 1 x 10^6 viable cardiomyocytes/ml using the number of viable cells/vial from the Certificate of Analysis (COA).

   Note: *Each Certificate of Analysis can be found online here: https://www.fujifilmcdi.com/coa-lookup/*

Plating iCell Cardiomyocytes^2 onto the FLX-96 plate

Note: *Timing is critical for plating iCell Cardiomyocytes^2 to avoid drying of the fibronectin-coated surface.*

1. Remove the fibronectin-coated FLX-96 plate from the cell culture incubator and work in a biological safety cabinet.

2. Aspirate the fibronectin from the whole FLX-96 plate.

3. Immediately dispense 100 µl of cell suspension (100,000 cells/well) to the center of each well using a multichannel pipettor.

   (optional) When using a VIAFLO ASSIST with a 12-channel pipette, transfer the cell suspension into a sterile reagent reservoir placed in the ViaFLO Assist, use program "CELLS_ADD100µL" and start the seeding procedure.

   Note: *Do not touch the membrane with the pipette tip, in order to prevent punctures of the silicone membranes.*

4. Culture the cells in iCell Cardiomyocytes Plating Medium in a cell culture incubator at 37°C, 5% CO_2 overnight to ensure cell adhesion to the silicone membranes.

   Note: *The freshly plated FLX-96 plate should be placed in a low traffic cell culture incubator and away from the door to minimize fluctuations in temperature and air movement in a low traffic incubator*

Culturing of iCell Cardiomyocytes^2 in the FLX-96 Plate

1. Equilibrate the iCell Cardiomyocytes Maintenance Medium to 37°C in a water bath prior to use.

   Note: *Aliquots of Maintenance Medium (22 ml per FLX-96 plate) may be removed from the stock bottle of Maintenance Medium.*

2. After overnight cell adhesion, replace 100% of the medium with 37°C iCell Cardiomyocytes Maintenance Medium. Hold the FLX-96 plate in a 45° angle and aspirate four rows of the FLX-96 plate (A-H) with a single channel vacuum aspiration system. Gently add 200 µl/well of 37°C iCell Cardiomyocyte Maintenance Medium to the side of each well to avoid disturbing the cardiomyocyte monolayer. Proceed with the next four rows of the FLX-96 plate (E – H) and repeat steps 2.

   (optional) When using a VIAFLO ASSIST with a 12-channel pipette, transfer the fresh medium into a sterile reagent reservoir and leave it right next to the VIAFLO ASSIST. Place an empty reagent reservoir in the VIAFLO ASSIST, use program "REMOVE100µL" and perform medium removal twice. Afterwards exchange the reagent reservoir containing the waste medium with the reagent reservoir containg the fresh medium and dispense the fresh medium with program "ADD100µL". Perform this step twice again to reach the final volume of 200µL per well.

   Note: *Avoid touching the bottom of the wells and rupture the silicone membranes when replacing the medium.*

3. Maintain the cardiomyocytes on the FLX-96 plate, replacing 100% of the spent medium with iCell Cardiomyocytes Maintenance Medium every 2 days.

4. Culture the cardiomyocytes in a cell culture incubator at 37°C, 5% CO_2.

5. Perform recordings from day 5 post plating when the cardiomyocytes have reached a stable baseline (Figure 1).

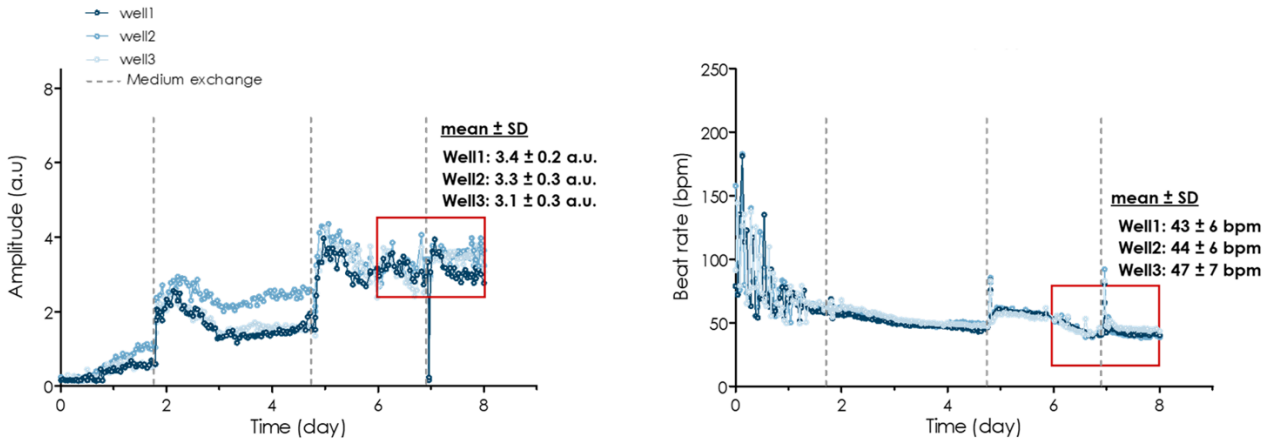


Figure 1: iCell Cardiomyocytes^2 form functional monolayers on FLX-96 plate. Amplitude (left) and beat rate (right) plots depict the formation of a confluent monolayer of beating cells on the FLX-96 plate. As shown, stable signal values are reached approximately 5 days post plating.

Data Acquisition and Analysis

Data Acquisition

Please refer to the CardioExcyte Control and DataControl96 Software manuals for complete instructions on how to acquire data on the instrument.

Compound Application

1. The day before compound addition, replace 100% of the medium with 37°C iCell Cardiomyocytes Maintenance Medium.

   Note: *Changing the iCell Cardiomyocytes Maintenance Medium about 18 hours (4 -8 hours when using serum-free medium) before compound treatment ensures that the cardiomyocytes have stabilized after medium replacement and that the medium volumes are uniform across the FLX-96 plate.*

2. Prepare stock solutions of test compounds in iCell Cardiomyocytes Maintenance Medium at a concentration of 10X the final concentration in a regular 96-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.*

3. Equilibrate the cell culture plate containing the 10X compound solutions (covered with a lid) in a cell culture incubator at 37°C, 5% CO_2

4. To begin the assay, quickly transfer 20 µl of the 10X compound solutions from the cell culture plate to the FLX-96 plate. Gently mix by pipetting 2-3 times.

   Note: *Beating rate and amplitude are temperature-dependent. It is recommended to add the compounds while the FLX-96 plate is placed on the FLEXcyte 96 system. If this is not possible, the FLEXcyte plate should not be kept outside the incubator for more than 2 minutes while compounds are added.*

Data Acquisition and Analysis

The CardioExcyte Control Software enables automatic beating detection of cardiomyocytes cultured on a FLX-96 plate placed onto the FLEXcyte 96 system. DataControl96 Software allows automated analysis of different contractility parameters before and after compound addition. Furthermore, DataControl96 Software enables the calculation of IC_{50} and EC_{50} of test compounds. See the CardioExcyte Control and DataControl96 Software manuals to discover all their features.

Representative Data

iCell Cardiomyocytes[2] were cultured on fibronectin-coated FLX-96 plate. At day 5, test compounds were applied. Compound effect on contractility amplitude, beat rate, pulse width, and compound-induced arrhythmias were recorded with CardioExcyte Control Software and analyzed with DataControl96 Software. The graphs displayed in Figure 2 were generated after the cardiomyocytes were exposed for 60 min to omecamtiv, nifedipine and dofetilide.

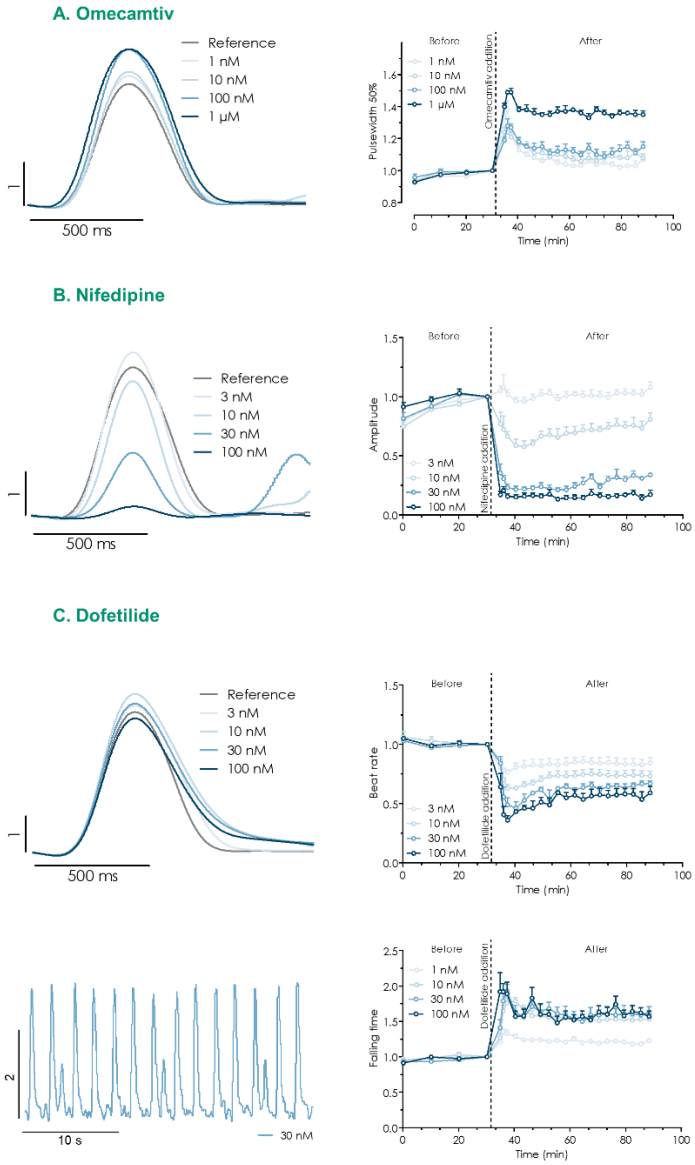


Figure 2: Compound effects on iCell Cardiomyocytes[2] contractility measured with the FLEXcyte 96 System ***A.*** *Omecamtiv, a positive inotropic agent, showed the expected amplitude increase and pulse width prolongation.* ***B.*** *Blocking $I_{Ca\text{-}L}$ with nifedipine induced a reduction of amplitude and increased the beat rate.* ***C.*** *Blocking I_{Kr} with dofetilide decreased the beat rate and prolonged the falling time. Dofetilide induced arrhythmic behavior, which was easily detected with the FLEXcyte 96 system.*

Summary

iCell Cardiomyocytes^2 equilibrate rapidly upon reanimation from cryopreservation to recapitulate native human cardiac myocyte physiology and function. The FLEXcyte 96 system is a reliable high throughput tool for *in vitro* cardiac contractility research, providing the user with data obtained under physiological conditions which resemble the native environment of human heart tissue. The methods and results described in this protocol highlight the ease of use with which robust and relevant data can be gathered on human cardiomyocyte contractility. The results obtained with the combination of iCell Cardiomyocytes^2 and the FLEXcyte 96 technology enable efficient and reliable results in preclinical drug development. In fact, the example FLEXcyte 96 data depicted in this protocol show drug-induced positive and negative inotropic effects that are consistent with the respective physiological responses in humans.

For questions about culturing our **iCell Cardiomyocytes^2**, please contact FCDI's Technical Support at

- fcdi-support@fujifilm.com
- +1 (877) 310-6688 (US toll-free) or +1 (608) 310-5100)

For inquiries about Nanion's **FLEXcyte platform**, please contact Nanion's Technical Support at

- Nanion HQ for general enquiries: info@nanion.de
- Nanion's Technical Support: support.cellular.networks@nanion.de

Revision History
Version 1.0: February 2021 AP-CM2FLEX11FEB2021