Cellular Dynamics international

iCell® Cardiomyocytes
Application Protocol

Performing Bioenergetic Analysis:
XF96 Extracellular Flux Analyzer

Introduction

The myocardium is the most metabolically active tissue in the body and is highly sensitive and responsive to increased cellular demands and environmental stimuli. Altered cellular bioenergetic events due to mitochondrial dysfunction have been implicated in cardiovascular pathologies, such as heart failure, ischemic and diabetic cardiomyopathy, and in drug-induced cardiac cytotoxicity mechanisms.

iCell® Cardiomyocytes, derived from human induced pluripotent stem cells, recapitulate biochemical, electrophysiological, mechanical, and pathophysiological characteristics of native human cardiac myocytes. Due to their human origin, high purity, and consistent functional relevance across manufacturing lots, iCell Cardiomyocytes represent an in vitro test system for cardiac biology interrogations in basic research and drug discovery, clinical cardiotoxicity prediction, and cardiac disease modeling.

The XF96 Extracellular Flux Analyzer (Seahorse Bioscience) is a non-invasive, label-free, high-throughput instrument that measures the metabolic activity of living cells by simultaneously monitoring mitochondrial respiration and glycolysis. iCell Cardiomyocytes can be cultured directly on an XF96 Cell Culture Microplate where energy metabolism can be modulated and analyzed. Together, iCell Cardiomyocytes and the XF96 Extracellular Flux Analyzer offer an in vitro platform for analyzing mitochondrial function, understanding pathophysiology, and evaluating therapeutic interventions in human cardiac myocytes.

This Application Protocol describes how to handle iCell Cardiomyocytes for use on the XF96 Extracellular Flux Analyzer and provides basic instructions for bioenergetic data acquisition and analysis.

Required Equipment, Consumables, and Software

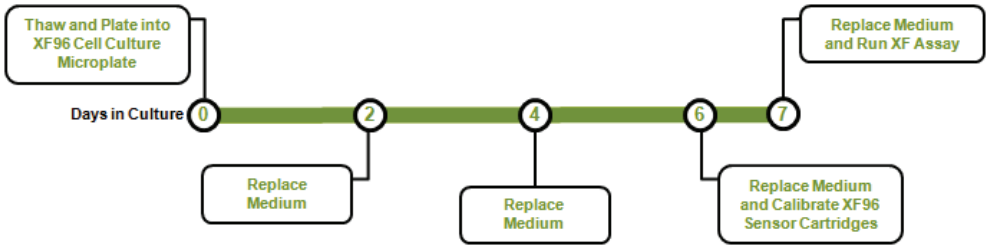
The following equipment, consumables, and software are required in addition to the materials specified in the iCell Cardiomyocytes User's Guide.

Item	Vendor	Catalog Number
Equipment		
8- or 12-well Multichannel Pipettor	Multiple Vendors	
XF96 Extracellular Flux Analyzer	Seahorse Bioscience	
Consumables		
iCell Cardiomyocytes Kit	Cellular Dynamics International (CDI)	CMC-100-110-001 CMC-100-110-005 CMC-100-010-001 CMC-100-010-005
XF Assay Medium	Seahorse Bioscience	100965-000
XF Calibrant Solution	Seahorse Bioscience	100840-000
XF Cell Mito Stress Test Kit	Seahorse Bioscience	101706-100
XF96 4-port FluxPak	Seahorse Bioscience	102310-001
Software		
XF96 Software	Seahorse Bioscience	

Notes

Workflow

iCell Cardiomyocytes are thawed and plated into an XF96 Cell Culture Microplate previously coated with gelatin. On days 2, 4, and 6 post-plating, spent medium is replaced with iCell Cardiomyocytes Maintenance Medium. On day 6 post-plating, XF96 Sensor Cartridges are calibrated. On day 7 post-plating, spent medium is replaced with complete XF Assay Medium, and the XF assay is performed.



Methods

Culturing iCell Cardiomyocytes

1. Coat an XF96 Cell Culture Microplate with 25 µl/well of 0.1% gelatin for at least 1 hour according to the iCell Cardiomyocytes User's Guide.
2. Thaw iCell Cardiomyocytes according to the User's Guide.

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3. Dilute the cardiomyocyte suspension in iCell Cardiomyocytes Plating Medium (Plating Medium) to 188,000 plated cells/ml. Refer to the User's Guide for instructions to calculate the *Target Plating Density* based on *Plating Efficiency*.
4. Aspirate the gelatin solution. Immediately add 80 µl/well of the cardiomyocyte suspension (15,000 plated cells/well).

   Note: *CDI recommends seeding the 60 inner wells of an XF96 Cell Culture Microplate to avoid edge effects. Add Plating Medium to all outer wells and all wells not containing cells to minimize the occurrence of edge effects.*

   Note: *If necessary, concentrate the cardiomyocytes by centrifuging at 180 x g for 5 minutes and resuspend to an appropriate concentration in Plating Medium.*
5. Place the XF96 Cell Culture Microplate in a biological safety cabinet at room temperature for 30 minutes to allow the cardiomyocytes to settle and ensure an even distribution.
6. Incubate in a cell culture incubator at 37°C, 7% CO_2.
7. Maintain the cardiomyocytes according to the User's Guide for 7 days, replacing the medium every other day (days 2, 4, and 6 post-plating).

Data Acquisition and Analysis

iCell Cardiomyocytes form a monolayer during the first 4 days after seeding into the XF96 Cell Culture Microplate. At day 7 post-plating, the preparation is suited for data acquisition. Refer to the manufacturer's instructions for the XF96 Extracellular Flux Analyzer to perform data acquisition and analysis.

Compound Application

1. Hydrate the XF96 Sensor Cartridge with 200 µl/well of XF Calibrant Solution approximately 24 hours before performing the metabolic assessment of iCell Cardiomyocytes using the XF assay.
2. Add the desired additives to the XF Assay Medium, if applicable, to create a complete XF Assay Medium on the day of the XF assay.
3. Adjust the pH of the complete XF Assay Medium to 7.4. Warm the complete XF Assay Medium to near 37°C.
4. Aspirate the spent medium from the XF96 Cell Culture Microplate.
5. Wash twice with 100 µl/well of complete XF Assay Medium approximately 2 hours before starting the XF assay.

6. Replace the complete XF Assay Medium with 120 µl/well of complete XF Assay Medium. Incubate the XF96 Cell Culture Microplate in a cell culture incubator at 37°C with atmospheric CO_2 levels (i.e., no additional CO_2) for 2 hours.

   Note: *It is critical to incubate the XF96 Cell Culture Microplate in atmospheric levels of CO_2 before the assay because CO_2 outgassing from the XF96 Cell Culture Microplate can affect the ECAR (Extracellular Acidification Rate) readout.*

   Note: *The total volume of 120 µl/well is optimal for use with the 4-port XF96 Sensor Cartridge. After injection of 20 µl of compound into each port, the final volume will be 200 µl/well.*

7. Load injection ports A, B, and C of the XF96 Sensor Cartridge with 20 µl of a 10X solution of components in the XF Cell Mito Stress Test Kit or a compound of interest, if applicable.

   Note: *It is important to load all ports. For wells not receiving a compound, load 20 µl/port of complete XF Assay Medium.*

Example Data

A representative respiratory profile of iCell Cardiomyocytes is shown in Figure 1 to exemplify the effects of modulating energy metabolism through the addition of compounds having a direct effect on mitochondrial integrity and function. Data were acquired using the XF96 Software set for a 5-minute mixing time and 2-minute measuring time. The basal mitochondrial respiration decreases after treatment with the ATP-synthase-inhibitor oligomycin. The oligomycin-insensitive respiration is due to proton leak. The subsequent addition of a proton ionophore, carbonyl cyanide 4-trifluoromethoxy phenylhydrazone (FCCP), uncouples oxidative phosphorylation from the electron transport system and shows the maximal respiration rate in iCell Cardiomyocytes. The mitochondrial electron transport chain is inhibited completely by the addition of the complex I-specific inhibitor rotenone. The graph also highlights the spare respiratory capacity, which corresponds to the ability of iCell Cardiomyocytes to respond to an increase in energy demand under oxidative stress conditions and mitochondrial dysfunction.

Notes

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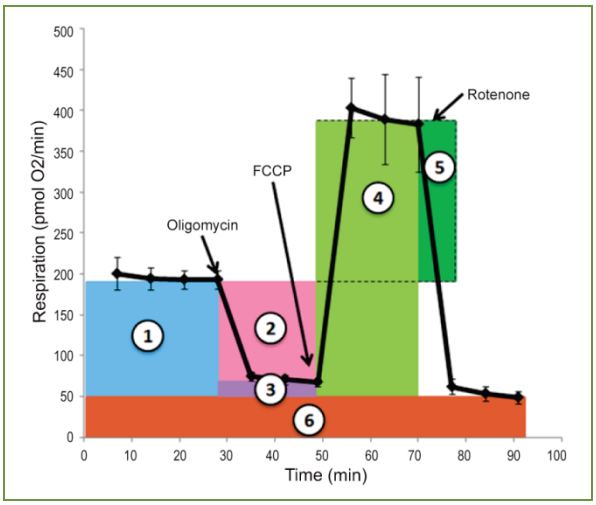

Figure 1: Representative Respiratory Profile of iCell Cardiomyocytes
iCell Cardiomyocytes were cultured on the XF96 Cell Culture Microplate for 7 days. The iCell Cardiomyocytes Maintenance Medium was replaced with XF Assay Medium supplemented with sodium pyruvate (1 mM), L-glutamine (4 mM), and galactose (10 mM) 2 hours before performing the XF assay. The data show: 1) basal respiration, 2) ATP production, 3) proton leak, 4) maximal respiration, 5) spare respiratory capacity, and 6) non-mitochondrial respiration. Oligomycin (1 µM), FCCP (0.5 µM), and rotenone (2 µM) were added where indicated.

Summary

iCell Cardiomyocytes provide an in vitro test system that recapitulates native human cardiac myocyte physiology and function while the XF96 Extracellular Flux Analyzer provides a label-free technology for non-invasive monitoring of basal oxygen consumption, glycolysis rate, ATP turnover, and spare respiratory capacity. The metabolic profile typical of cardiac myocytes can be monitored, and the treatment effects on energy metabolism can be detected and quantified. The methods and data presented here highlight how to obtain robust and relevant data with respect to the cellular bioenergetic function and responses to oxidative stress in living human cardiomyocytes.

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