Performing Bioenergetic Analysis:
Seahorse XFe96 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® Cardiomyocytes², 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 Cardiomyocytes² represent an in vitro test system for cardiac biology interrogations in basic research and drug discovery, clinical cardiotoxicity prediction, and cardiac disease modeling.

The Seahorse XFe96 Analyzer (Agilent Technologies) 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 Cardiomyocytes² can be cultured directly on an XF96 Cell Culture Microplate where energy metabolism can be modulated and analyzed. Together, iCell Cardiomyocytes² and the Seahorse XFe96 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 Cardiomyocytes² for use on the Seahorse XFe96 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 Cardiomyocytes² User's Guide.

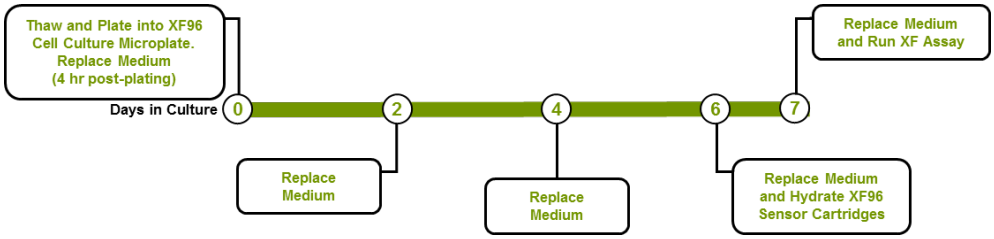
Item	Vendor	Catalog Number
Equipment		
8- or 12-well Multichannel Pipettor	Multiple Vendors	
Seahorse XFe96 Analyzer	Agilent Technologies	
Consumables		
iCell Cardiomyocytes² Kit	Cellular Dynamics International (CDI)	CMC-100-012-000.5 CMC-100-012-001
Seahorse XFe96 FluxPak	Agilent Technologies	102601-100 102416-100
Seahorse XF Base Medium	Agilent Technologies	102353-100

Item	Vendor	Catalog Number
Seahorse XF Cell Mito Stress Test Kit	Agilent Technologies	103015-100
Software		
Wave Controller	Agilent Technologies	

Notes

Workflow

iCell Cardiomyocytes² are thawed and plated into an XF96 Cell Culture Microplate previously coated with gelatin. At 4 hours post-plating and every 48 hours thereafter, spent medium is replaced with iCell Cardiomyocytes Maintenance Medium (Maintenance Medium). On day 6 post-plating, XF96 Sensor Cartridges are hydrated. On day 7 post-plating, spent medium is replaced with assay medium, and the XF assay is performed.



Methods

Thawing iCell Cardiomyocytes²

1. Coat an XF96 Cell Culture Microplate with 50 µl/well of 0.1% gelatin for at least 1 hour according to the iCell Cardiomyocytes² User's Guide.
2. Calculate the final volume of iCell Cardiomyocytes Plating Medium (Plating Medium) needed to obtain a final cell plating density of 250,000 viable cells/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 80 µl/well of the cardiomyocyte suspension (20,000 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.*
5. Place the XF96 Cell Culture Microplate in a biological safety cabinet at room temperature for 20 - 30 minutes to allow the cardiomyocytes to settle and ensure an even distribution.
6. Incubate in a cell culture incubator at 37°C, 5% CO_2 for 4 hours.

Notes

Culturing iCell Cardiomyocytes[2]

1. Aspirate the spent Plating Medium at 4 hours post-plating and replace with 80 µl/well of Maintenance Medium.
2. Maintain the cardiomyocytes according to the User's Guide for 7 days, replacing medium every other day (days 2, 4, and 6 post-plating).

Data Acquisition and Analysis

iCell Cardiomyocytes[2] form a monolayer and start to synchronously beat during the first 4 days after plating into the XF96 Cell Culture Microplate. On day 7 post-plating, the preparation is suited for data acquisition. Refer to the manufacturer's instructions for the Seahorse XFe96 Analyzer to perform data acquisition and analysis.

Compound Application

1. Hydrate the XF96 Sensor Cartridge with 200 µl/well of XF Calibrant Solution at approximately 24 hours before performing the metabolic assessment of iCell Cardiomyocytes[2] using the XF assay.
2. Add the desired additives to the XF Base Medium, if applicable, to create a complete assay medium on the day of the XF assay.
3. Adjust the pH of the assay medium to 7.4 and equilibrate it to approximately 37°C.
4. Aspirate the spent medium from the XF96 Cell Culture Microplate.
5. Wash twice with 200 µl/well of assay medium at approximately 45 - 60 minutes before starting the XF assay.
6. Add 180 µl/well of 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 45 - 60 minutes.

   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 volume of 180 µl/well is the recommended starting volume for the Constant Concentration protocol described in the manufacturer's instructions for the Seahorse XFe96 Analyzer.*
7. Load injection ports A, B, and C of the XF96 Sensor Cartridge with the recommended volumes of components of the XF Cell Mito Stress Test Kit (or a compound of interest, if applicable) according to the Constant Concentration protocol described in the manufacturer's instructions for the Seahorse XFe96 Analyzer.

Example Data

Representative respiratory profiles of iCell Cardiomyocytes² are 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 Wave Controller software set for a 3-minute mixing time and 3-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 Cardiomyocytes². The mitochondrial electron transport chain is inhibited completely by the addition of the complex I-specific inhibitor rotenone and the cytochrome c reductase-inhibitor antimycin A.

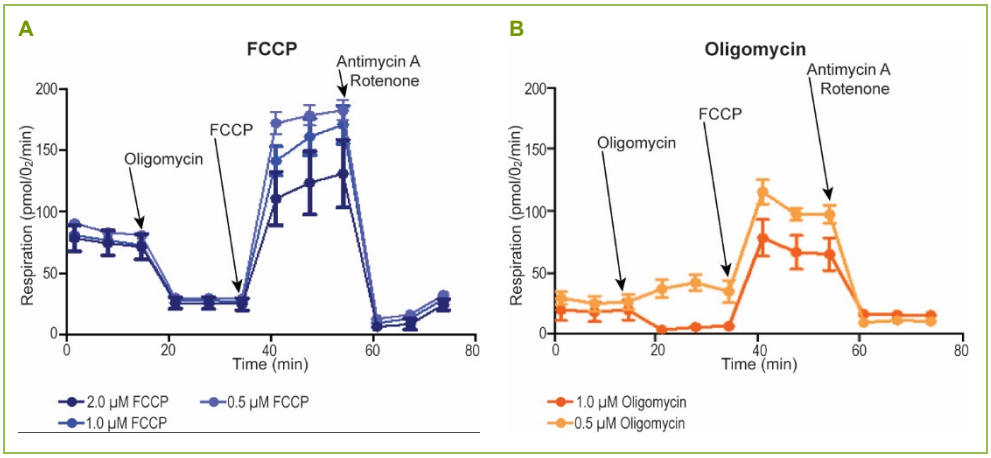


Figure 1: Representative Respiratory Profile of iCell Cardiomyocytes²
iCell Cardiomyocytes² were cultured on the XF96 Cell Culture Microplate for 7 days. The Maintenance Medium was replaced with XF Base Medium (pH 7.4) supplemented with sodium pyruvate (1 mM), L-glutamine (2 mM), and glucose (10 mM) 45 - 60 minutes before performing the XF assay. Optimal concentration of oligomycin (1 µM), FCCP (0.5 µM), antimycin A (0.5 µM), and rotenone (0.5 µM) were added where indicated. Panels A and B show the concentration effect on the respiratory profile for FCCP and oligomycin, respectively.

Notes

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Summary

iCell Cardiomyocytes[2] provide an in vitro test system that recapitulates native human cardiac myocyte physiology and function while the Seahorse XFe96 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.

Revision History
Version 1.0: December 2016
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