FUJIFILM
Cellular Dynamics

iCell® Cardiomyocytes²
Application Protocol

# Measuring Cardiac Activity:

*Functional Maturation on the xCELLigence RTCA ePacer*

## Introduction

iCell Cardiomyocytes² have been optimized for rapid recovery from cryopreservation. Their derivation from human induced pluripotent stem cells, high purity, functional relevance and ease of use 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 ePacer is a scalable system that allows for functional maturation of iCell Cardiomyocytes² by providing electrical pacing with less stress than currently available models. By controlling the electrical activity, organized sarcomeric structure improves and the cells demonstrate a positive force frequency relationship which increases the accuracy of measurements, well to well consistency and experimental alliance. The system provides a high-throughput assay platform compatible with many options of relevant readouts for safety/toxicology assessment and drug discovery.

This Application Protocol describes how to handle and perform pacing using the iCell Cardiomyocytes² on the xCELLigence RTCA ePacer for functional maturation.

## 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** | | |
| Multichannel Pipettor, 20 and 200 µl | Multiple Vendors | |
| xCELLigence RTCA CardioECR | ACEA Biosciences, A part of Agilent Technologies | |
| xCELLigence RTCA ePacer | ACEA Biosciences, A part of Agilent Technologies | |
| **Consumables** | | |
| iCell Cardiomyocytes² Kit, 01434 | FUJIFILM Cellular Dynamics, Inc. | R1017<br>CMC-100-012-000.5 |
| Centrifuge Tubes, 50ml Sterile | Multiple Vendors | |
| Dulbecco's Phosphate Buffered Saline without $Ca^{2+}$ and $Mg^{2+}$ (DPBS) | ThermoFisher Scientific | 14190 |
| E-Plate CardioECR 48 | ACEA Biosciences, A part of Agilent Technologies | 00300600940<br>00300601110 |
| Fibronectin | Roche | 11051407001<br>11080938001 |
| Microcentrifuge Tubes | Multiple Vendors | |
| Sterile Reagent Reservoirs | Multiple Vendors | |
| Sterile Water | Multiple Vendors | |

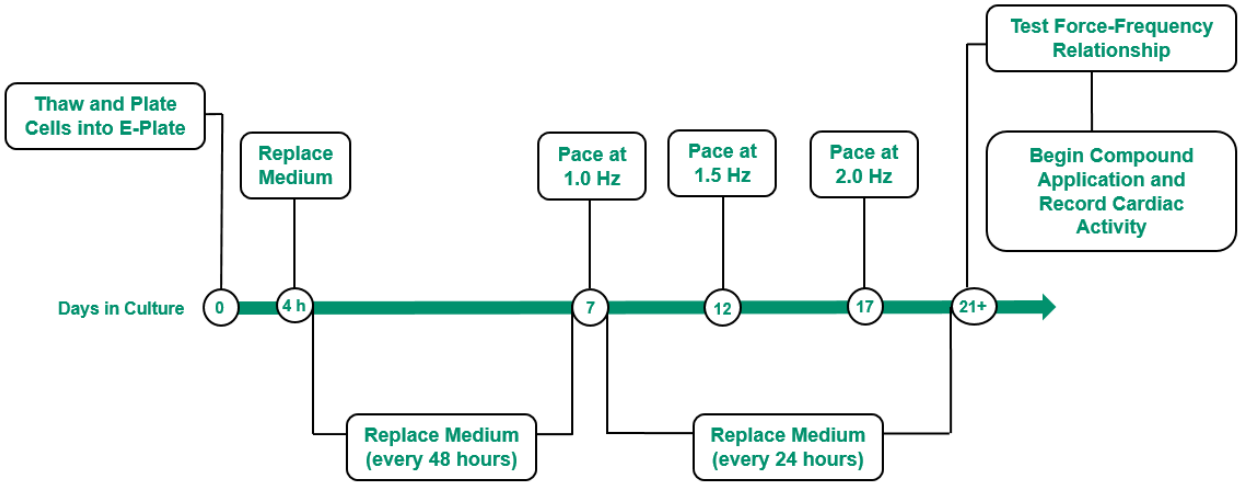
| Software | |
|---|---|
| RTCA CardioECR Data Acquisition and Analysis Software | ACEA Biosciences, A part of Agilent Technologies |
| RTCA ePacer Instrument Software | ACEA Biosciences, A part of Agilent Technologies |

## Workflow

The cardiomyocytes are thawed and plated into an E-Plate previously coated with fibronectin. Plating Medium is replaced with Maintenance Medium 4 hours after plating and Maintenance Medium is replaced every 48 hours thereafter.

Electrical stimulation (pacing) of the cardiomyocytes begins on day 7 at 1.0 Hz and increases by 0.5 Hz every 5 days. During pacing, Maintenance Medium is replaced every 24 hours.

On day 22, force-frequency relationship is measured. Cells can be treated with compounds and cardiac activity recorded when the force-frequency relationship is positive.




## Methods

**Note:** Prior to beginning, place the RTCA CardioECR and RTCA ePacer stations in a cell culture incubator at 37°C, 5% $CO_2$.

### Preparing the E-Plate - Day 1

1. Reconstitute fibronectin in sterile water at 1 mg/ml according to the manufacturer's instructions.
2. Dilute 1 mg/ml fibronectin solution in DPBS to a final concentration of 10 µg/ml.
3. Add 50 µl/well of the 10 µg/ml fibronectin solution to the center of the wells of the E-Plate to evenly coat the bottom of the well.
4. Incubate at 37°C for at least 1 hour.

### Thawing Cardiomyocytes- Day 1

1. Equilibrate iCell Cardiomyocytes Plating Medium to 37°C.
2. Aspirate the fibronectin solution from the E-Plate.

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

3. Add 50 µl/well of Plating Medium to the center of the wells.
4. Equilibrate the E-Plate in a cell culture incubator at 37°C, 5% $CO_2$ for at least 15 minutes.
5. Transfer the E-Plate the RTCA CardioECR station. Record a background measurement according to the RTCA CardioECR Instrument Operator's Guide.
6. Insert the E-Plate into RTCA ePacer station. Record a background measurement according to the RTCA ePacer Instrument Operator's Guide.
7. Thaw the cardiomyocytes according to their User's Guide to a final volume of 4 ml. Dilute the 1 ml cell suspension from the cryovial in 1 ml of Plating Medium rinse and an additional 2 ml of Plating Medium.
8. Use the viable cells/vial from the Certificate of Analysis to calculate the final volume of Plating Medium needed to obtain a final cell plating density of 1 x $10^6$ viable cells/ml.
9. Remove a sample of cells to confirm viability using a hemocytometer (using trypan blue dye exclusion). If the cell viability is below 50%, contact Technical Support for assistance.
10. Dispense 50 µl/well of the cell suspension (50,000 cells/well) to the center of each well of the E-Plate.
11. Leave the E-Plate undisturbed at room temperature for approximately 30 minutes to allow the cells to settle.
12. Culture the cardiomyocytes in a cell culture incubator at 37°C, 5% $CO_2$ for 4 hours.
13. Equilibrate an aliquot of Maintenance Medium to 37°C.
14. Replace the Plating Medium with Maintenance Medium. Tilt the E-Plate, remove the spent medium and gently add 100 µl/well of Maintenance Medium to the side of the well to avoid disturbing the cells.
15. Culture the cardiomyocytes in the RTCA ePacer station at 37°C, 5% $CO_2$.

    ***Note:*** *Inserting the E-Plate into the RTCA ePacer station allow the cells to be continuously monitored. The E-Plate can also be inserted into the RTCA CardioECR station for monitoring.*

### **Maintaining Cardiomyocytes in the E-Plate:** Days 1 - 6

1. Equilibrate an aliquot of Maintenance Medium in a 37°C water bath immediately before use.
2. Maintain the cardiomyocytes on the E-Plate by replacing 100% of the spent medium with Maintenance Medium every 48 hours (100 µl/well).
    a. Tilt the E-Plate to remove medium and gently add medium down the side of the well to avoid disturbing the cells.
3. Culture the cardiomyocytes in the RTCA ePacer at 37°C, 5% $CO_2$.

### **Pacing the Cardiomyocytes at 1.0 Hz:** Day 7

*Do not allow the pipettor tips to touch the bottom of the well during medium removal or addition. Tilt the E-Plate to remove medium and gently add medium down the side of the well to avoid disturbing the cells.*

1. Equilibrate an aliquot of Maintenance Medium to 37°C.
2. Replace 100% of the spent medium with Maintenance Medium (100 µl/well).

a. Tilt the E-Plate to remove medium and gently add medium down the side of the well to avoid disturbing the cells.

3. Insert E-Plate into the RTCA ePacer.
4. Initiate pacing by applying an electrical stimulus with a frequency of 1.0 Hz, pulse amplitude of 1.1 V and pulse width of 0.4 ms.

   ***Note:*** *The electrical stimulus must contain a sufficient combination of sweeps and intervals to pace for at least 120 hours (e.g. 25 sweeps of 8 hour intervals).*

5. Maintain the cardiomyocytes on the E-Plate by replacing 100% of the spent medium with Maintenance Medium every 24 hours.
6. Culture the cardiomyocytes in the RTCA ePacer at 37°C, 5% $CO_2$ for 5 days.

**Pacing the Cardiomyocytes at 1.5 Hz:** Day 12

1. Equilibrate an aliquot of Maintenance Medium in a 37°C water bath.
2. Abort the previously applied electrical stimulus.
3. Replace 100% of the spent medium with Maintenance Medium (100 µl/well).

   a. Tilt the E-Plate to remove medium and gently add medium down the side of the well to avoid disturbing the cells.

4. Insert E-Plate into the RTCA ePacer.
5. Initiate pacing by applying an electrical stimulus with a frequency of 1.5 Hz, pulse amplitude of 1.1 V and pulse width of 0.4 ms.

   ***Note:*** *The electrical stimulus must contain a sufficient combination of sweeps and intervals to pace for at least 120 hours (e.g. 25 sweeps of 8 hour intervals).*

6. Maintain the cardiomyocytes on the E-Plate by replacing 100% of the spent medium with Maintenance Medium every 24 hours.
7. Culture the cardiomyocytes in the RTCA ePacer in a cell culture incubator at 37°C, 5% $CO_2$ for 5 days.

**Pacing the Cardiomyocytes at 2 Hz:** Day 17

1. Equilibrate an aliquot of Maintenance Medium in a 37°C water bath immediately before use.
2. Abort the previously applied electrical stimulus.
3. Replace 100% of the spent medium with Maintenance Medium (100 µl/well).

   a. Tilt the E-Plate to remove medium and gently add medium down the side of the well to avoid disturbing the cells.

4. Insert E-Plate into the RTCA ePacer.
5. Initiate pacing by applying an electrical stimulus with a frequency of 2.0 Hz, pulse amplitude of 1.1 V and pulse width of 0.4 ms.

   ***Note:*** *The electrical stimulus must contain a sufficient combination of sweeps and intervals to pace for at least 120 hours (e.g. 25 sweeps of 8 hour intervals).*

6. Maintain the cardiomyocytes on the E-Plate by replacing 100% of the spent medium with Maintenance Medium every 24 hours.
7. Culture the cardiomyocytes in the RTCA ePacer in a cell culture incubator at 37°C, 5% $CO_2$ for 5 days.

### Measuring the Force-Frequency Relation of the Cardiomyocytes: Day 21+

1. Culture the cardiomyocytes in the RTCA ePacer at 37°C, 5% $CO_2$ without electrical stimulus for 4 hours to allow the culture to resume spontaneous beating rhythms.
2. Record a spontaneous beating measurement according to the RTCA ePacer Instrument Operator's Guide.
3. Pace the cells at 1.0 Hz for 1 sweep with a 10 minute interval. Enable the Auto function to perform the recording at the end of the step.
4. Pace the cells at 1.5 Hz for 1 sweep with a 10 minute interval. Enable the Auto function to perform the recording at the end of the step.
5. Pace the cells at 2.0 Hz for 1 sweep with a 10 minute interval. Enable the Auto function to perform the recording at the end of the step.
6. Evaluate the slope of the force-frequency relationship.
    a. If the slope is positive, proceed to Data Acquisition and Analysis.
    b. If the slope is negative, continue to apply electrical stimulus with a frequency of 2.0 Hz, pulse amplitude of 1.1 V and pulse width of 0.4 ms for approximately 24 hours before reevaluating the force-frequency relationship.

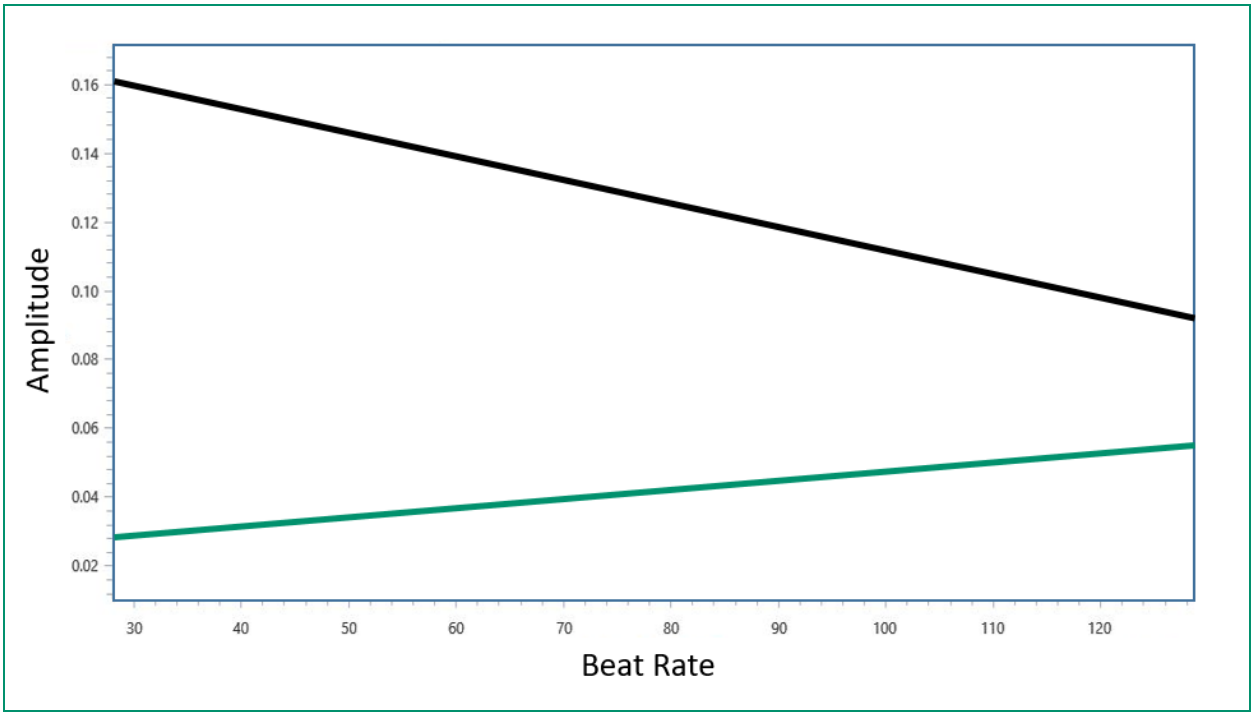



**Figure 1: Representative Force-Frequency Relationship**
*iCell Cardiomyocytes[2], 01434 exhibit a negative force frequency relationship (black) at day 7 post-thaw. After pacing, the force-frequency relationship is positive (green) at day 22 post-thaw.*

## Data Acquisition and Analysis

### Applying Compounds

1. Equilibrate an aliquot of Maintenance Medium to 37°C.

2. Remove the spent medium and gently add 90 µl/well of Maintenance Medium to the side of the well to avoid disrupting the monolayer.
3. Culture the cardiomyocytes in the RTCA CardioECR or ePacer at 37°C, 5% $CO_2$ without electrical stimulus to allow the culture to resume spontaneous beating rhythms.

   ***Note:*** *Spontaneous beating rhythms may not resume for up to 4 hours.*
4. Monitor the activity of the E-Plate to ensure a regular beating rhythm and stable whole-peak amplitudes are reached.
5. Prepare test compounds in Maintenance Medium at 10X the final concentration in a 96-well cell culture plate.

   ***Note:*** *Final DMSO concentrations above 0.1% should be used with caution. Therefore the 10X compound solutions should not exceed 1% DMSO.*
6. Equilibrate the plate containing the 10X compound solutions at 37°C, 5% $CO_2$ for at least 1 hour.
7. Quickly transfer 10 µl of the 10X compound solutions to the appropriate wells of the E-Plate.

   ***Note:*** *Beat rate and amplitude are temperature dependent. Do not keep the E-Plate outside the incubator for more than 5 minutes while compounds are added.*
8. Return the E-Plate to the RTCA CardioECR station. Record measurements according to the RTCA CardioECR Instrument Operator's Guide.

### Data Acquisition and Analysis using the RTCA Software

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

## Example Data

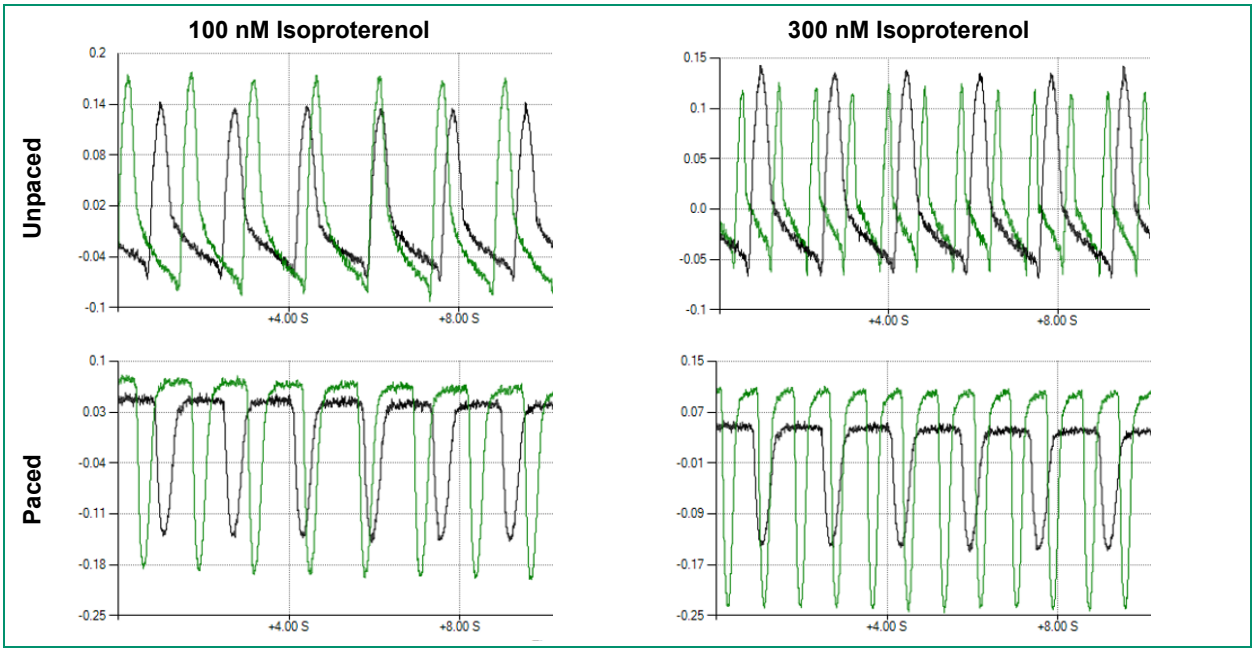



**Figure 2: Representative Effects of Long-term Electrical Pacing on Impedance**
*Paced iCell Cardiomyocytes[2], 01434 display the expected positive chronotropic response to Isoproterenol depicted by increased beat frequency and increased amplitude (green) when compared to a vehicle control (black).*

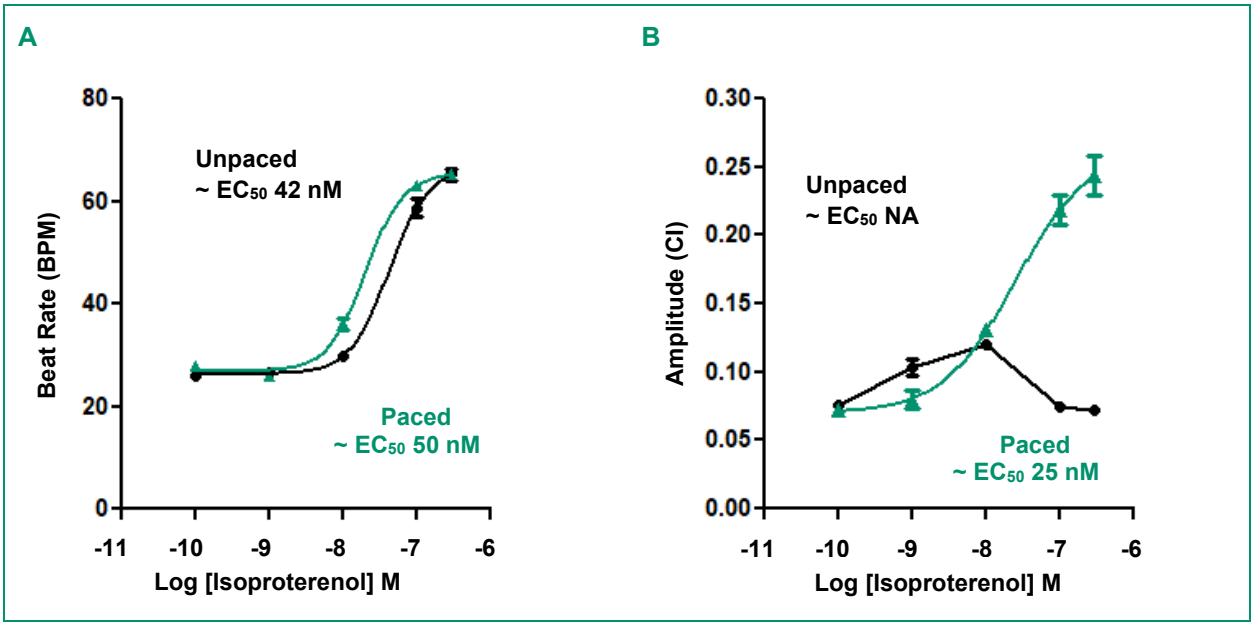



**Figure 3: Representative Effects of Electrical Pacing on Isoproterenol Response**

*iCell Cardiomyocytes[2], 01434 dose response curves for ß-adrenergic agonist isoproterenol is concentration dependent. Panel A shows the expected positive chronotropic response when stimulated with isoproterenol. Panel B shows electrical pacing reverses the negative inotropic response to isoproterenol resulting in a greater than 3-fold increase in amplitude.*



**Revision History**

Version 1.1: December 2019