

User Guide for iCell Cardiomyocytes Assay on an xCELLigence RTCA CardioECR System

Introduction

The Agilent xCELLigence RTCA CardioECR system enables noninvasive, label-free, real-time monitoring of cardiomyocytes. This system can evaluate viability, contractility, and electrical activity involved in excitation-contraction (EC) coupling across the cardiomyocyte monolayer. The system works well with all cardiomyocytes, but this process has been optimized specifically for analysis using the iCell cardiomyocyte.

Note: This process is specifically for direct plating of one unit of iCell cardiomyocytes.

Reagents, materials, and equipment

Equipment

- xCELLigence RTCA CardioECR system (Agilent, p/n 380601210)
- CO₂ incubator (multiple vendors)
- Laminar flow hood (multiple vendors)
- 8-channel multichannel pipettor, 20 and 200 µL (multiple vendors)

Reagents, cell lines, and consumables

- E-Plate CardioECR 48 (Agilent, p/n 300600940)
- iCell cardiomyocytes kit (Cellular Dynamics International)
- Fibronectin (FN) stock (Sigma-Aldrich, Cat. no. F1141)
- Dulbecco's phosphate buffered saline without Ca²⁺ and Mg²⁺ (DPBS) (multiple vendors)

Detailed process

Day 1: experiment setup

1. Coating the E-Plate CardioECR 48 (approximately 1.5 hours).
 - a. Dilute the fibronectin (FN) stock 1:100 in sterile DPBS to a final concentration of 10 µg/mL.
 - b. Transfer 50 µL of diluted FN to each well of the E-Plate CardioECR 48.
 - c. Incubate the coated E-Plate CardioECR 48 at 37 °C for at least 1 hour or 4 °C overnight.
2. Measure the background (approximately 5 to 10 minutes).
 - a. Replace the coating buffer in the E-Plate CardioECR 48 with 50 µL of prewarmed iCell Cardiomyocytes Plating Medium (Plating Medium).
 - b. Insert the E-Plate CardioECR 48 into the RTCA CardioECR station inside a CO₂ incubator, according to the Cardio ECR system Operator's Guide.
 - c. Start the RTCA CardioECR data acquisition software and click the Layout tab. Select the sample wells by highlighting the area in the well map corresponding with the sample wells. Enter the requested experimental information and click Apply.
 - d. Click the Schedule tab, then add Step 1 for the background measurement process.

Caution: Sweep number, interval, and duration settings for Step 1 are preset for background measurements and should not be changed.
 - e. Click the Start/Continue icon to obtain the background reading. The background reading step will take 1 second.
 - f. Remove the E-Plate CardioECR 48 from the station and place in a laminar flow hood for cell seeding.
3. Thaw, count, and seed iCell cardiomyocytes on the E-Plate CardioECR 48 (approximately 1.5 hours).
 - a. See the CDI iCell cardiomyocytes User Guide on thawing and counting cells. Seed 20,000 cells/well in an E-Plate CardioECR 48.
 - b. After cell seeding, leave the E-Plate CardioECR 48 in the laminar flow hood for 30 minutes at room temperature to ensure even cell distribution in the wells.

Caution: 30 minutes incubation at RT is critical for a successful cell culture as demonstrated by reliable cell performance during the assay.
 - c. Transfer the seeded E-Plate CardioECR 48 to the incubator. Allow 15 minutes for temperature equilibration before inserting the plate into the RTCA CardioECR station.

Caution: Temperature equilibration is recommended for preventing condensation on the bottom of the E-plate CardioECR 48, which will interfere with accurate assessment of the field potential signal.

- d. Click the Schedule tab and add Step 2 to monitor cell attachment and growth (optional).
Recommended schedule step:
 - 1) 100 sweeps of 20-second durations (extra sweeps can be aborted)
 - 2) Six-hour interval between each sweep
 - 3) Sampling rate:
Impedance: 2 or 12.8 ms
ECR: 0.1 ms (10 kHz)
- e. Click the Start/Continue icon to start Step 2.
4. Replacing medium (approximately 10 minutes).
 - a. Plating Medium should be replaced with iCell Cardiomyocyte Maintenance Medium (Maintenance Medium) 48 hours after seeding. Incubate the Maintenance Medium in a 37 °C water bath.
Caution: It is important to use warm medium for the medium change.
 - b. Pause the RTCA CardioECR system monitoring by clicking the Pause button in the RTCA CardioECR software. Remove the E-Plate CardioECR 48 from the CardioECR Station and transfer the plate to a laminar flow hood. Slightly tilt the plate, and using a multichannel pipette, gently remove the plating media completely from the wells.
 - c. Slowly add 100 μ L of the equilibrated media by tilting the E-Plate CardioECR 48 at an angle and adding the medium to the side of the well.
Caution: Do not disturb the cell monolayer during the medium change.
 - d. Bring the E-Plate CardioECR 48 back to the incubator. After 15 minutes of temperature equilibration, engage the E-Plate CardioECR 48 into the CardioECR station and resume measurements by clicking the Start/Continue icon.
 - e. The regular medium change should be performed every 48 hours during the culture.

Tip: The beating signal of the iCell cardiomyocytes should be detectable within 96 to 144 hours after cell seeding. However, beating frequency will be variable after switching from plating medium to culture medium. It becomes more regular 10 days post-seeding.

Days 2 to 14: cell maintenance

1. Cell culture maintenance (approximately 14 days).
 - a. Replace the iCell cardiomyocytes maintenance medium every 48 hours. Follow the procedure "Replacing medium" for medium change.
 - b. Monitor the beating activity and field potential performance daily until the iCell cardiomyocytes duplicate a synchronous beating profile. The coefficient of variation (CV) of beating period during each recording should be less than 10%.

Days 14 to 16: cell status quality control (QC) before compound addition

1. Measure cell beating and field potential signals (4 hours).
 - a. If the medium needs to be refreshed on cell status QC day:
 - a. Equilibrate the iCell culture medium in a 37 °C water bath.
 - b. Replace culture medium following the steps described in the “Replacing medium” section.
 - c. Incubate cells on the station for at least 4 hours before taking measurements for QC assessment.
 - d. Click the Schedule tab to add Step 3 for cell status QC purposes.

Recommended schedule step for cell status QC measurement:

- 1) Five sweeps of 20-second durations
- 2) One-hour interval between each sweep
- 3) Sampling rate:

Impedance: 2 or 12.8 ms

ECR: 0.1 ms (10 kHz)

- b. If no medium change is needed on cell status QC day:

Recording can be performed immediately.

Recommended schedule step for cell status QC measurement:

- 1) Five sweeps of 20-second durations
- 2) One-hour interval between each sweep
- 3) Sampling rate:

Impedance: 2 or 12.8 ms

ECR: 0.1 ms (10 kHz)

Cell status quality control

Cardio mode test (beating activity) recommended conditions:

Condition	Recommended Reading	Recommended CV Across Different Recording Time Points
Beating Rate (BR)	BR >20 beats/min	CV <15%
Beating Amplitude (Amp)	Amp ≥0.07	CV <15%
Beating Rhythm Irregularity (BRI)	BRI <0.1	

Note: A stable cardio signal is normally achieved before a stable FP signal occurs.

ECR mode test (electrical activity) recommended conditions:

Condition	Recommended Reading	Recommended CV Across Different Recording Time Points
Firing Rate (FR)	FR >20 times/min	CV <15%
Sodium Spike Amplitude (Amp)	a. 90% of electrodes detect >0.3 mV of sodium spike b. 90% of electrodes have consistent amplitude of sodium spike	CV <15%

Day 17: compound addition

1. Cell culture preparation (10 minutes).

At least 4 hours before compound addition, replace the culture media with 90 μL of fresh media equilibrated to 37 °C.

Note: Evaporation rates can vary across the E-Plate CardioECR 48. Changing the maintenance medium before compound treatment is required to ensure uniform medium volumes across the E-Plate.

2. Measure baseline (35 minutes).

Measure baseline of beating activity before compound treatment. Click the Schedule tab to add the next step (Step 4) for the baseline measurement of beating and electrical activity.

Recommended schedule step for baseline measurement:

- 1) Seven sweeps of 60-second durations

- 2) Five-minute interval

- 3) Sampling rate:

Impedance: 2 or 12.8 ms

ECR: 0.1 ms (10 kHz)

3. Compound preparation and dilution (approximately 1 hour)

- a. Determine the predicted effective concentration ranges for the test substances.

- b. Prepare stock solutions of test compounds by dissolving the compounds in appropriate solvent. If DMSO is used as the solvent, dissolve the compound in a high stock concentration, if possible, (ideally 1000-fold of the highest test concentration) and store at $-20\text{ }^{\circ}\text{C}$.

- c. Thaw the compound stock solutions and prepare 1000x concentrated serial dilutions of compounds in appropriate solvent (DMSO or H_2O).

- d. Transfer solvent diluted compounds to the wells of a V bottom microplate for further dilution in the culture medium (to a final concentration of 10x).

4. Compound addition: Add the diluted compound and monitor for a short-term (<1 hour) or long-term (24 hours) period.

- a. Set up steps for compound testing Step 5 and Step 6 in the Schedule section, and make sure the Auto reading box between Step 5 and Step 6 is checked.

Recommended schedule step for short-term monitoring (<1 hour) (Step 5)

- 1) 13 sweeps of 60-second durations

- 2) Five-minute intervals

- 3) Sampling rate:

Impedance: 2 or 12.8 ms

ECR: 0.1 ms (10 kHz)

Recommended schedule step for long-term monitoring (<24 hours) (Step 6)

- 1) 24 sweeps of 60-second durations
- 2) One-hour interval between each sweep
- 3) Sampling rate:

Impedance: 2 or 12.8 ms

ECR: 0.1 ms (10 kHz)

- c. Make sure to enter compound names and tested concentrations in the layout page.
- d. Before removing cells from the incubator for compound treatment, pause the experiment first, then disengage the E-Plate CardioECR 48 from the RTCA CardioECR station. Transfer the plate with cells onto the stabilization core of the Temperature Tool to minimize temperature fluctuation.
- e. Carefully, but quickly, add the compound to the cells. Using a multichannel pipette, transfer 10 μ L of the compounds diluted in the culture medium from the V bottom microplate to the E-Plate CardioECR 48.

Caution: Include wells treated with the solvent only at the same final concentration as compound-treated wells for the negative control.

Caution: It is important to have a multichannel pipette, tips, and tip waste box ready. This will ensure the minimum time for adding compounds and exposing cells to the ambient temperature and oxygen levels outside of the tissue culture incubator.

- f. Insert the E-Plate CardioECR 48 into the CardioECR station. Start the next step immediately.

Caution: To capture all the information on electrical and beating activity changes after compound addition. The process of compound addition should not exceed 3 to 5 minutes. Ensure that all recording schedules are preprogrammed to help minimize this time.