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Product Information

Cor.At® GFP-i-xCELLigence Cardiomyocyte Kit

Catalog Number **AXIO0063**

TECHNICAL BULLETIN

Product Description

Cor.At® cells are cardiomyocytes derived from transgenic mouse induced pluripotent stem (miPS) cells. These cells are puromycin resistant and have the green fluorescent protein (GFP) reporter gene controlled by the promoter of *Myh6*, which encodes α-myosin heavy chain (αMHC).[1]

Cor.At cells are produced by *in vitro* differentiation of mouse induced pluripotent stem (iPS) cells and puromycin selection of cardiomyocytes. Similar to primary cells, they have limited proliferative capacity, demonstrated by BrdU incorporation assays. These cells express cardiac-specific connexin-43, which demonstrates the potential for electric coupling. Patch clamp analyses, as well as multi-electrode array recordings, demonstrate normal electrophysiological properties. Further, transplantation of stem cell-derived cardiomyocytes into infarcted heart resulted in teratoma-free engraftment.[2]

These cardiomyocytes have many advantages over primary cells and cell lines. These cells are highly standardized and 100% pure when cultured with puromycin. The culture and maintenance of the cells require minimal laboratory time when compared to the culture and maintenance of rat neonatal cells. Reproducible results can be expected for every assay.

Features of the cells:

- 100% pure with fully functional cardiomyocyte phenotype
- Pre-qualified lots ensure reproducible results
- Frozen, stored, and thawed with complete recovery of functionality
- Part of an entire *in vitro* based system
- Absolutely no fibroblast contamination
- Express all relevant cardiac ion channels (K^+, Ca^{2+}, and Na^+) and show predictive response to cardiotoxic compounds when compared to clinical data

The RTCA Cardio Instrument is a label-free, real-time system for dynamic monitoring of cardiomyocyte beating and assessment of cardiotoxicity. Microelectronic cell sensors are fabricated in the bottom of a 96 well plate (E-Plate® Cardio 96) in which the cardiomyocytes are cultured.

Using a high data acquisition rate of 12.5 milliseconds, the instrument can resolve the changes in impedance induced by the contraction/relaxation cycle of the cardiomyocytes. Changes in beating and rhythmicity induced by compound treatment are indicative of possible cardiotoxic side effects, e.g., induction of arrhythmia or Torsades-des-Pointes.[3,4]

Components

Cor.At GFP-i xCELLigence Cardiomyocytes (Mouse iPS cell derived) 4M (Cor.At GFP-iC-4M) 4×10^6 mouse iPS cell derived cells Catalog Number AXIO0013	1 vial
Cor.At Culture Medium Catalog Number AXIO0076	2×250 mL
Cor.At Fibronectin-Based Coating Catalog Number AXIO0101	55 µL
Cor.At xCELLigence Sub-Kit Catalog Number AXIO0110 Set contains the following:	1 set
• Disposable Neubauer Hemacytometer Catalog Number AXIO0099	1 each
• Trypan Blue Solution Catalog Number AXIO0093	50 µL

Reagents and Equipment Required but Not Provided.

- Puromycin solution, Catalog Number AXIO0078
- PBS without Ca^{2+} and Mg^{2+}
- E-Plate Cardio 96 (Roche Diagnostics, Catalog Number 06417051001)
- Real-Time Cell Analyzer (RTCA) Cardio Instrument (Roche Diagnostics)
- Biological safety cabinet certified for Level I handling of biological materials
- Incubator with humidity and gas control to maintain 37 °C and 95% humidity in an atmosphere of 5% CO_2 in air
- 37 °C water bath
- Inverted fluorescence microscope with Green Fluorescent Protein (GFP) or Fluorescein Isothiocyanate (FITC) filters
- Sterile 50 mL polypropylene (PP) tubes
- Centrifuge with rotor for 50 mL PP tubes (not required for 96 well plate format)
- 8 channel or 12 channel micropipette
- Sterile pipette tips
- Sterile reagent reservoirs, Catalog Number CLS4870

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The cardiomyocytes (Catalog Number AXIO0013) are shipped in liquid nitrogen dry shippers and stored at –196 °C.

AXIO0076 is shipped on dry ice and storage at –20 °C is recommended.

AXIO0101 ships at ambient temperature and may be stored at 2–8 °C.

AXIO0099 and AXIO0093 ship at ambient temperature and may be stored at room temperature.

Procedure

Cor.At cells are genetically modified mouse cells and should be handled according to local directives (Typically Biosafety level 1).

The cardiomyocytes should be cultured using sterile cell culture techniques and good laboratory practices.

Cells can be inactivated by autoclaving at 121 °C for 20 minutes.

Day 0 - Thawing of Cor.At Culture Medium

Thaw the Cor.At Culture Medium (Catalog Number AXIO0076) and the Puromycin (Catalog Number AXIO0078) at 4 °C overnight or at room temperature (15–25 °C).

Notes: Do not attempt to thaw the medium at 37 °C because precipitation of the proteins in the medium may occur.

The culture medium contains light sensitive compounds and should be kept in the dark while thawing.

Day 1 – Coating of E-Plate Cardio 96 with Fibronectin

1. Dilute the Fibronectin-Based Coating (Catalog Number AXIO0101) 1:100 with PBS without Ca^{2+} and Mg^{2+}. Mix thoroughly.

2. Transfer 50 µL of the diluted fibronectin solution into each well of the E-Plate Cardio 96. Be careful to not touch the bottom of the plate.

3. Place the freshly coated E-Plate Cardio 96 into the 37 °C incubator for 3 hours or overnight at 2–8 °C.

Day 1 – Media Preparation

1. Pipette 25 mL of thawed Culture Medium into a sterile 50 mL PP tube, warm to 37 °C before use.
   Note: This medium is used for the background measurement and for thawing the cells.

2. Pipette 8 mL of thawed Culture Medium into a second sterile 50 mL PP tube and warm to 37 °C before use.
   Note: This medium is used to wash the cells once the cells are thawed.

3. Prepare Culture Medium containing puromycin – Pipette 50 mL of the thawed Culture Medium (Catalog Number AXIO0076) into a sterile 50 mL PP tube, and add 50 µL of the Puromycin solution (Catalog Number AXIO0078). Mix well and store at 4 °C and warm to 37 °C before use.
   Note: Use this medium for the first medium change.

4. Store the remaining Culture Medium (Catalog Number AXIO0076) without puromycin at 4 °C. The remaining medium is used for medium changes **after 48 hours of culture** and can be kept at 4 °C for up to three weeks.
   Note: It is highly recommended to note the date when the Culture Medium (Catalog Number AXIO0076) bottle was opened to ensure the medium is not used if the bottle has been opened for more than three weeks.

Day 1 – Program the RTCA

1. Open the RTCA Cardio Software and click on the *Layout* tab. Enter requested information about the cells, cell number, compounds, and compound dilutions.

2. Click the *Schedule* tab. Add *Step_1* (background step), which by default is preset to 0 second duration, 1 sweep, and 1 interval.
   Note: Do not change the preset parameters required for background calculation.

3. Add *Step_2* and enter the parameters for *Step_2*. This step is designed to monitor cell attachment and growth, and for assessing beating activity at regular intervals. Enter 100 sweeps at 1 hour intervals with a sweep duration of 10 seconds.

4. Add *Step_3* to measure beating baseline directly before compound addition. These data will be used to normalize the values after compound addition. Measure for a minimum of 10 intervals with 20 seconds duration, every minute. Enter 20-100 sweeps at 1 minute intervals for the sweep duration of 20 seconds. Once the compounds are ready to be added, this Step can be aborted.

5. Add *Step_4* to measure fast response of the cells after compound addition. Measure for 100 intervals with 20 seconds duration, every minute.

6. Add *Step_5* for long-term measurement. Enter 100 sweeps. Increase the interval to 15 minutes or 1 hour. Continue to measure with a sweep duration of 20 seconds or decrease the sweep duration to 10 seconds. Tick the *Auto* checkbox for *Step_5* to directly continue with Step_5 after Step_4 is finished.

Day 1 – Background Measurement of Coated E-Plate Cardio 96

1. Carefully aspirate the fibronectin coating solution and immediately fill each well with 180 µL of culture medium from the tube containing 25 mL of medium (Media preparation, step 1). Incubate the plate for at least 5 minutes at 37 °C.
   Note: For the background measurement, you have to fill **all** wells with medium, regardless of the number of wells to be used in the experiment.

2. Place the E-Plate Cardio 96 on the RTCA Cardio Station inside the CO_2 incubator.

3. Click on the *Start/Continue* icon to obtain the background reading.

4. After the background reading is finished, remove the E-Plate Cardio 96 from the RTCA Cardio Station and place in the laminar flow hood until time of cell seeding.

Day 1 – Washing & Counting Cells

1. Add 50 µL of Trypan Blue solution (Catalog Number AXIO0093) to 40 µL of PBS without Ca^{2+} and Mg^{2+} in a microcentrifuge tube.

2. Prepare the hemacytometer by cleaning the chambers and coverslip with isopropanol. Wipe the hemacytometer dry using lint-free tissue.

3. Remove the vial containing the cardiomyocytes from the shipment box or storage. Immediately place the vial in a 37 °C water bath for two minutes. When a small ice clump is still visible, remove the vial from the water bath and wipe with 70% isopropanol or ethanol to sterilize.

4. Immediately pipette the cell suspension into the second 50 mL tube containing 8 mL of thawed Culture Medium **without puromycin** (Media preparation, step 2) to wash the cells.

5. Use 1 mL of thawed Culture Medium **without puromycin** from the first 50 mL tube to rinse the vial, ensuring any cells remaining in the vial are also recovered. Add this volume to the 50 mL tube (there should now be ~10 mL of cell suspension in the tube).

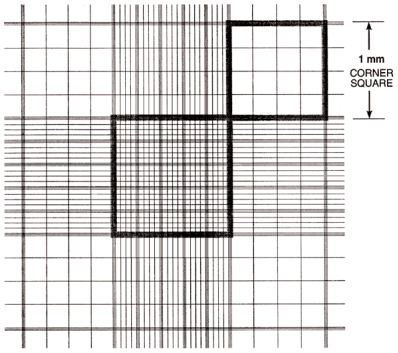
6. Centrifuge cells at 200 × *g* for 5 minutes at room temperature.

7. Aspirate the supernatant, leaving the cell pellet intact. In preparation for cell counting, resuspend the cells in 1 mL of Culture Medium without puromycin.

8. Pipette 10 µL of the cell suspension into the prepared Trypan Blue/PBS solution, mix well, and incubate for 5 minutes at 37 °C (i.e., place tube into the incubator). This is a 10-fold dilution of the cells.

9. After 5 minutes, fill the Neubauer hemacytometer with the cell suspension (step 8), ensuring the chambers are not underfilled or overfilled (use 10 µL of cells/Trypan Blue/PBS suspension). Count both the clear, refractile cells and the blue colored cells in the 4 large outer quadrants. This is the **total number of cells (N)** in the four large, outer quadrants (see Figure 1).

Figure 1.
Neubauer Hemacytometer – showing dimensions of each square.



10. Subsequently, count the **number of blue cells (D)** in the same four, large outer quadrants. These are the non-viable cells that have incorporated the Trypan Blue dye. Because of the characteristic decrease in cell membrane integrity in non-viable cells, the Trypan Blue dye is able to pass through the cell membrane.

11. Calculate the average number of total cells (A_N) per square by dividing N by 4 (N/4) and the average number of non-viable cells (A_D) per square by dividing D by 4 (D/4).

$$A_N = N \div 4$$
$$A_D = D \div 4$$

12. Calculate the **absolute number of viable cells (V)** using the formula:

$$V = (A_N - A_D) \times 10 \times 10^4 \times 0.5 \text{ mL}$$

$$\text{Cell count per mL} = \text{Average cell count per square} \times \text{Dilution factor} \times 10^4$$

Day 1 – Plating Cells

1. Adjust the cell concentration to ~1×10^6 viable cells per mL.

2. For the entire 96 well plate, mix 14.8 mL of Culture Medium **without puromycin** with 4.2 mL of the cell suspension (step 1, ~1×10^6 viable cells/mL). Mix the cell suspension carefully by pipetting up and down.
   Note: The 19 mL of cell suspension (40,000 cells/ 180 µL, total of 3.84×10^6 viable cells) is sufficient volume for seeding the entire plate (17.28 mL [96 wells × 180 µL/well] plus ~2 mL of dead volume for the reagent reservoir).

3. Transfer the cell suspension to the sterile reagent reservoir.

4. Aspirate the medium from the E-Plate Cardio 96 and immediately transfer 180 µL/well of the cell suspension using a manual 8-channel pipette. Each well of the E-Plate is seeded with 40,000 viable cells in 180 µL of medium.

5. **Do not move the plate**, let it stand in the laminar flow hood for 30 minutes before moving it into the RTCA Cardio Instrument in the incubator. This step is important to achieve an even distribution of the cells. Thereafter, carefully transfer the plate to the RTCA Cardio Instrument and start measurement by pressing the *Start/Continue* icon to start step_2.

Day 2 – First Medium Change with Culture Medium containing puromycin
Exchange the culture medium in the morning, 16–18 hours after seeding.

1. Pre-warm the 50 mL of Culture Medium **containing** puromycin to 37 °C and transfer it to a sterile reagent reservoir.

2. Pause the RTCA Cardio Instrument monitoring by pressing the Pause button in the RTCA Cardio Software. Disengage the E-Plate Cardio 96 from the RTCA Cardio Station and transfer it to the laminar flow hood.

3. Remove 90 µL of the medium from every well with an 8-channel pipette. Remove the medium from the top, being careful not to touch the bottom of the plate with the pipette tip and disturbing the cell layer. (When using an automatic pipette, be sure to use the slowest pipetting rate; when using a manual pipette, pipette up and down slowly). Add 90 µL of Culture Medium containing puromycin to each well using an 8-channel pipette at slowest speed. Repeat this step 4 times to achieve an almost complete medium change in the wells.
   Note: It is important to perform the medium change in this manner, as it minimizes the effect of the medium change on the cells. The cells are very sensitive and should not be left without medium at any time. While changing the medium, the cells usually stop beating for a moment, this is a temperature effect rather than a medium change effect.

4. Place the E-Plate Cardio 96 back into the Cardio Station as quick as possible and resume the measurement by clicking the *Start/Continue* icon.

Day 2 – Second Medium Change with Culture Medium without puromycin
In the afternoon (~8 hours after the first medium change), repeat the medium change as described but use Culture Medium (Catalog Number AXIO0076) without puromycin pre-warmed to 37 °C.

Day 3 – Medium Change
Perform the medium change early in the morning (not more than 16–18 hours after the last medium change) using the previously described procedure with Culture Medium (Catalog Number AXIO0076) without puromycin.

Day 3 – Compound Addition
1. Compounds can be added 1–2 hours after medium change. Observe the well graph to determine if the cells are beating regularly and with acceptable amplitude for use in compound analysis (see Table 1).

Table 1.
Acceptance Criteria for Cells at timepoint 0

Total Cell Index	>5
Beating Frequency	75–150 beats per minute
Amplitude	>0.05

Each well has to meet the criteria to be included in data evaluation.

2. Program the steps for compound measurement (see Table 2). Other measurement times can be used if necessary.

Table 2.
Programming the RTCA software for compound measurement

Step	Sweep Duration	Speed (ms)	Sweeps	Interval	Unit	Comments
3	20 s	12.9	100	2	minute	Baseline
4	20 s	12.9	4	5	minute	Compound addition
5	20 s	12.9	1	15	minute	
6	20 s	12.9	3	1	minute	0.5 h 1×
7	20 s	12.9	1	87	minute	
8	20 s	12.9	3	1	minute	2 h 3×
9	20 s	12.9	1	2	hour	
10	20 s	12.9	3	1	minute	4 h 3×
11	20 s	12.9	1	2	hour	
12	20 s	12.9	3	1	minute	6 h 3×
13	20 s	12.9	7	1	hour	
14	20 s	12.9	3	1	minute	12 h 3×
15	20 s	12.9	7	2	hour	
16	20 s	12.9	3	1	minute	
17	20 s	12.9	3	1	hour	Medium change
18	20 s	12.9	3	1	minute	Wash out

3. Set up the compound stock solutions: Dissolve compounds in Cor.At medium at 2× the final concentration used in the respective well. See Figure 2 for a standard plate layout with 4 compounds at 5 concentrations each.
   Note: The final concentration of DMSO must not exceed 0.1%.

Figure 2.
Layout of an E-Plate Cardio for compound testing (4 compounds, 5 conc. each)

	1	2	3	4	5	6	7	8	9	10	11	12
A	DMSO	10 µM	1 µM	100 nM	10 nM	1 nM	Ctr.I	10 µM	1 µM	100 nM	10 nM	1 nM
B	DMSO	10 µM	1 µM	100 nM	10 nM	1 nM	Ctr.I	10 µM	1 µM	100 nM	10 nM	1 nM
C	DMSO	10 µM	1 µM	100 nM	10 nM	1 nM	Ctr.I	10 µM	1 µM	100 nM	10 nM	1 nM
D	DMSO	10 µM	1 µM	100 nM	10 nM	1 nM	Ctr.I	10 µM	1 µM	100 nM	10 nM	1 nM
E	Ctr.II	10 µM	1 µM	100 nM	10 nM	1 nM	DMSO	10 µM	1 µM	100 nM	10 nM	1 nM
F	Ctr.II	10 µM	1 µM	100 nM	10 nM	1 nM	DMSO	10 µM	1 µM	100 nM	10 nM	1 nM
G	Ctr.II	10 µM	1 µM	100 nM	10 nM	1 nM	DMSO	10 µM	1 µM	100 nM	10 nM	1 nM
H	Ctr.II	10 µM	1 µM	100 nM	10 nM	1 nM	DMSO	10 µM	1 µM	100 nM	10 nM	1 nM

Compound I
Compound II
Compound III
Compound IV
Control I (e.g., Isoproterenol) → frequency modulation
Control II (e.g., E4031) → arrhythmia induction

4. Use a 96 deep well plate mimicking the layout of the E-plate for compound dilutions. Incubate the prepared double concentrated compound solutions at 37 °C and 5% CO_2 for at least 15 minutes.
5. In the meantime, start the baseline measurement (step 3) in the RCTA Cardio Software.
6. Directly before adding the compounds, abort the baseline measurement, disengage the E-Plate Cardio 96 from the RCTA Cardio Station, and transfer it to the laminar flow hood. Also transfer the deep well plate to the laminar flow hood.
7. From the E-Plate, remove 90 µL of medium per well using an 8 channel pipette. Immediately transfer 90 µL/well of the respective compound solution from the deep well plate into each well of the E-Plate using an 8-channel pipette, following the layout scheme (see Figure 2).
   Note: Pipette with slow speed and do not touch the bottom of the well with the pipette tip.
8. Place the E-Plate Cardio 96 back into the Cardio Station as quick as possible and start steps 4 and 5 for immediate and medium term measurement.

Day 4: Wash out

1. 24 hours after the compound addition, the RTCA Cardio software stops the measurement automaticlly after step 17.
2. Prewarm Cor.At medium without Puromycin to 37 °C.
3. Disengage the E-Plate Cardio 96 from the RCTA Cardio Station and transfer it to the laminar flow hood.
4. Replace the compound solutions with fresh Cor.At medium (step 2).
5. Place the E-Plate Cardio 96 back into the Cardio Station as quick as possible and start step 18 for wash out measurement.
6. After step 19 the experiment is finished.

References

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