Application of Axol iPSC-Derived Cardiomyocytes on the xCELLigence® RTCA Cardio (ACEA Biosciences)

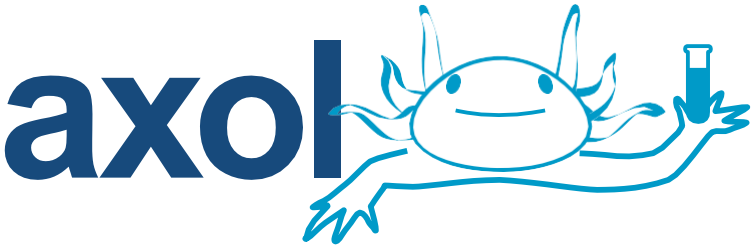
With additional drug application protocol

Experts' Protocol Series

Application Protocol

Kindly provided by Dr J Gill, Durham University

UK



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Materials Required

1. Fibronectin
2. Phosphate buffered saline (containing Ca^{2+} and Mg^{2+})
3. ACEA Biosciences - E- plate® cardio 96
4. Axol Human iPSC-Derived Cardiomyocytes (4x ax2505; Total $4x10^6$ cells)
5. Axol Cardiomyocyte Maintenance Medium Kit (ax2530-500)
6. Serum (FBS)
7. Trypan blue

Preparing Complete Cardiomyocyte Maintenance Medium

1. Thaw the Cardiomyocyte Maintenance Medium and its supplement **overnight at 4 ºC**.
2. Add the supplement to the basal medium to make the complete medium. For long-term storage, we recommend preparing aliquots of the **Complete Cardiomyocyte Maintenance Medium** and storing at **-80 ºC**.
3. Before use pre-warm an aliquot of **Complete Cardiomyocyte Maintenance Medium** in a water bath at **37 ºC**.

Preparing Cardiomyocyte Plating Medium

1. Take an aliquot of **Complete Cardiomyocyte Maintenance Medium** and add 10% serum (FBS). This is termed **Plating Medium**.

Methods:

Coating of the E-Plate® Cardio 96:

Day 0:

1. Fibronectin coating solution (10 µg/mL) was prepared by diluting **60 µL** of fibronectin stock (1 mg/mL) into **6 mL** Phosphate Buffered Saline (containing Ca^{2+} and Mg^{2+}).
2. To each well of the E-plate® cardio 96, **50 µL** of fibronectin solution (10 µg/mL) was added.
3. The plate was then sealed and incubated overnight at **4 °C**

Determination of Plate Background Impedance:

Day 1:

1. **50 mL** of Axol plating medium (containing 10 % serum) was warmed to **37 °C**
2. Excess fibronectin solution was removed from each well of the plate by aspiration
3. To each well of the plate, **180 µL** of warmed plating medium was added, and the plate transferred to the CO_2 incubator for **30 min** to equilibrate to **37 °C**
4. The plate was transferred into the xCELLigence® RTCA cardio, within the CO_2 incubator
5. A background impedance measurement was conducted (as per xCELLigence® RTCA cardio protocol)
6. The plate was kept in the incubator until required for cell seeding

Plating Axol iPSC-Derived Cardiomyocytes:

Four vials of Axol Human iPSC-Derived Cardiomyocytes ($4x10^6$ cells) were removed from liquid nitrogen storage and quickly transferred on ice to cell culture laboratory.

All vials were thawed rapidly in a **37 °C** water bath, with constant swirling to assist uniform thawing.

1. Once only a small ice clump is evident, the vials were transferred to a sterile laminar flow hood
2. The contents of all four vials were transferred into a sterile centrifuge tube containing **6 mL** warm plating medium, producing a total volume of **10 mL**
3. The cell suspension was centrifuged at 200 x g for **5 min** and the resulting

cell pellet carefully resuspended in **10 mL** fresh plating medium

4. **10 µL** of cell suspension was mixed with **10 µL** trypan blue solution and the number of viable cells calculated using a haemocytometer and light microscopy

Example Calculation: [Haemocytometer cell count] x10^4 x 2(dilution) x10 (total volume)
= ***Total Number of Cells***

5. The concentration of cells was adjusted by addition of the relevant volume of plating medium to obtain between **$2.4x10^6$** and **$3.0x10^6$** cells in a total volume of **18 mL**
6. The plate was removed from the incubator and transferred to the laminar flow hood. All media was then aspirated from the plate
7. To each well, **180 µL** of cell suspension was added to produce a total of between 24000 and 30000 cells per well
8. The plate was left in the laminar flow hood for **30 min** to allow the cells to settle and distribute and attach within the wells of the plate.
9. The plate was then transferred to the xCELLigence® RTCA cardio (within the incubator) and impedance readings automatically recorded according to the required protocol (ideally at least every **30 min**) throughout the study

Note: Seeding density is dependent and may need to be optimized accordingly for your experiments. However, a maximum of 3000 cells per well is recommended

Maintenance of Axol iPSC-Derived Cardiomyocytes on the xCELLigence® RTCA Cardio

Medium replenishment:

1. Media was changed once daily throughout the duration of the study
2. Culture Medium was warmed to **37°C**
3. The instrument was paused and the plate transferred to the laminar hood (in transfer module)
4. From each well, **90 µL** of medium was removed and replaced with **90 µL** of fresh warmed media. This was repeated three times.
5. Once all wells had been replenished, the plate was returned to the xCELLigence® RTCA cardio and the study continued

Media Change:

1. Cells were maintained in plating medium until cell growth kinetics demonstrated a plateau, indicative of cell coverage of the well and cessation of proliferation (cell index 5.5, approximately 72-96 hours post-plating)
2. Once cell index stabilisation was achieved, all plating media was carefully removed from the plate and replaced with the basal medium (no added serum)
3. Daily media changes were continued, using the methodology previously described.

Application - Drug Evaluation:

1. After stabilised synchronous beating was observed (approximately 24-72 hours post media change), a range of investigational drugs were added to evaluate drug response.
2. As a means of evaluating cardiomyocyte responsiveness and reactivity, a range of concentrations of isoproterenol (β-adrenergic receptor agonist) and carvedilol (β-adrenergic receptor antagonist) were added to selected wells.

Results:
From Drug Evaluation Study

The background reading of all wells within the E-plate® cardio 96 was within the acceptable value range and therefore available for addition of cells and study evaluation.

Post-plating, all wells were confirmed to contain cells, with impedance readings strongly indicating viable attachment of cells to the fibronectin.

Initial Growth

During the post-seeding period in Axol plating medium, an increase in impedance (as determined by cell index) was measured in all wells within the plate (Figure 1). This increase was indicative of cellular growth and continued viability, with a plateau and cessation of growth reached by 93 hours (see Figure 1).

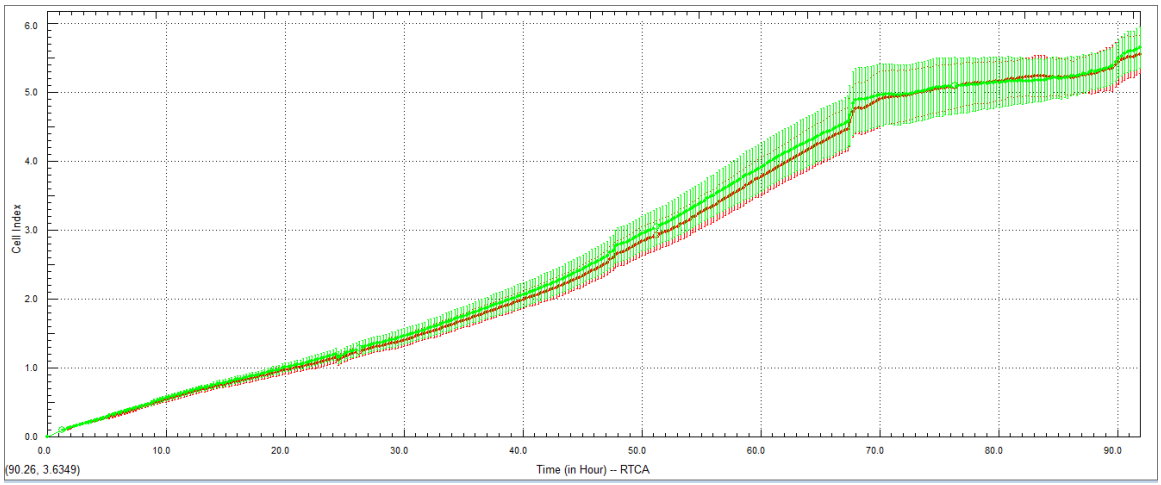

Figure 1: Increased cell index of Axol iPSC-derived cardiomyocytes over time. Each well contained 24000 cells incubated in Axol plating medium. Exponential cell growth was demonstrated over the initial 93-hour post-seeding period. Cell index (impedance) values plateaued by 93 hours, indicative of confluency and cessation of cellular proliferation.

All wells were monitored for evidence of contractility and sustainability of this phenotype throughout subsequent phases of the study. [*For ease of interpretation and presentation, although all wells were monitored, the same four wells are depicted in all of the subsequent figures of this report. The full dataset is available for interpretation if required.]*

At 24-hours post-plating, an indication of a primitive attempt of cellular contractility was observed (Figure 2A), with indicative contractility observed by 48 hours (Figure 2B).

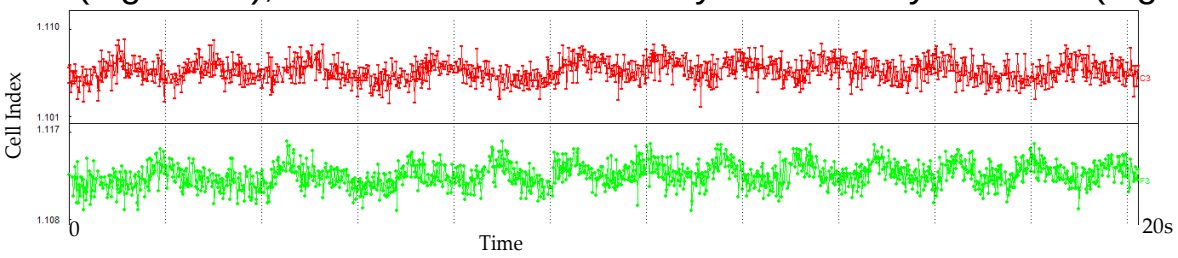

***Figure 2A**:* Primitive asynchronous cell contraction at 24-hours post plating

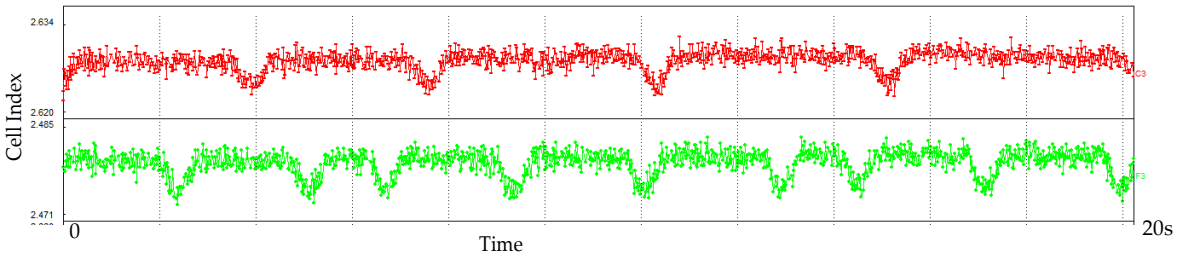

***Figure 2B**:* Indicative evidence of cellular contractility at 48-hours post plating

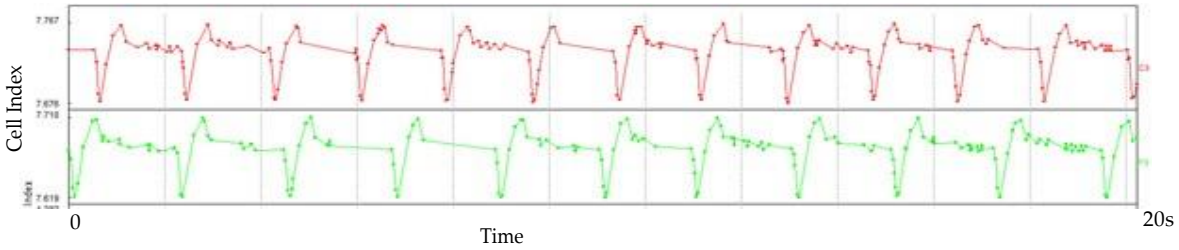

***Figure 2C**:* Indicative evidence of synchronous cell contractility at 120-hours post plating

Evaluation of Drugs Upon Cardiomyocyte Contractility

At approx. 265h a range of concentrations of isoproterenol (β-adrenoceptor agonist) and carvedilol (β- adrenoceptor agonist) were added to selected wells and the effects of beating of cells grown in both medium were observed (Figure 3).
The Axol iPSC-Derived Cardiomyocytes responded to both isoproterenol and carvedilol in the expected manner and in a dose-dependent fashion. This strongly indicates the 'clinical' relevance of these cells and their utility for drug screening applications.

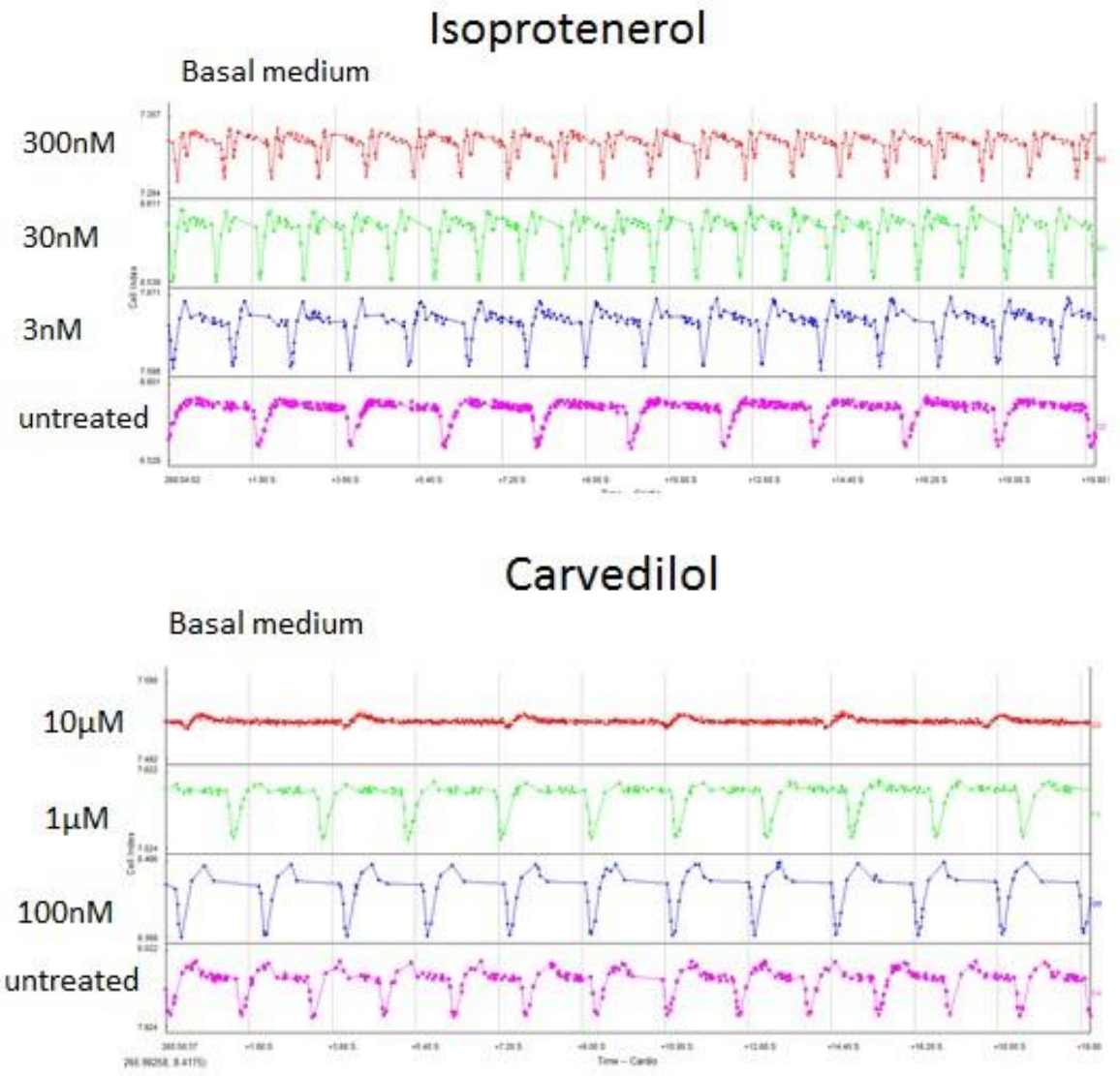

Figure 3: Effect of isoproterenol and carvedilol upon contractility of Axol iPSC-Derived Cardiomyocytes

Conclusions

- Axol iPSC-Derived Cardiomyocytes are suitable for use on the ACEA Biosciences xCELLigence® RTCA cardio.
- E-plate® cardio 96 (specific for us in the xCELLigence® RTCA cardio) are required to be pre-coated in fibronectin for use with Axol iPSC-Derived Cardiomyocytes
- Initial plating of the cells in the E-plate® cardio 96 requires the use of a serum-rich **plating medium**
- Axol iPSC-Derived Cardiomyocytes demonstrate potential for excitation-contraction in the **plating medium**, but with limited synchronicity and scope for longer-term study
- Culture of Axol iPSC-derived cardiomyocytes in **Basal Medium** is conducive for longer-term cell survival and viability
- Culture of Axol iPSC-derived cardiomyocytes in **Basal Medium** is permissive of excitation-contraction coupled cell contractility, with a clearly defined 'full contractile shape' and a clinically relevant contractile phenotype
- Axol iPSC-Derived Cardiomyocytes respond to cardiac drugs and therefore have potential for use in cardiotoxicity and cardiomyocyte-pharmacology studies

Technical Support

- ## Online Resources

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  For more information or technical assistance, call +44 (0) 1223 497 119, or email support@axolbio.com. US Toll Free Tel: 1-800-678-2965 (1-800-678-AXOL), US Toll Free Fax: 1-800-861-2965 (1-800-861-AXOL).

  Where we are:
  Axol Bioscience Ltd, Suite 3, The Science Village, Chesterford Research Park, Little Chesterford, CB10 1XL, UK, +44 1223 751 051

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