

# Human iPS cell-Derived Cardiomyocytes ReproCardio2<sup>TM</sup> as an Analytic Tool for Large-scale Toxicity Screening

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## Abstract

The force-frequency relationship in cardiac muscle contraction is different in developmental maturation and pathological states. Hence, cardiac contraction can be utilized for the evaluation of chemical compounds especially when these compounds affect the beating rate.

In this study, various chemical stimulations on the contraction of human iPS cellderived cardiomyocytes ReproCardio2<sup>TM</sup> were examined with electrophysiological assay as well as calcium-imaging. ReproCardio2<sup>TM</sup> possess stable BPM and regular QT interval with minimum differences between cell-lots after been thaw from cryopreservation. With the addition of chemical compounds, the extension or reduction of QT-interval can be obtained by both electrophysiological assay and calcium-imaging. Other novel utility of **ReproCardio2<sup>TM</sup>** has also been developed. Taken together, ReproCardio2<sup>TM</sup> is useful for analyzing the effects of chemical compounds on contractile function. Therefore, ReproCardio2<sup>TM</sup> can be utilized in large-scale screening of large-scale of chemical compounds



### Materials and Methods

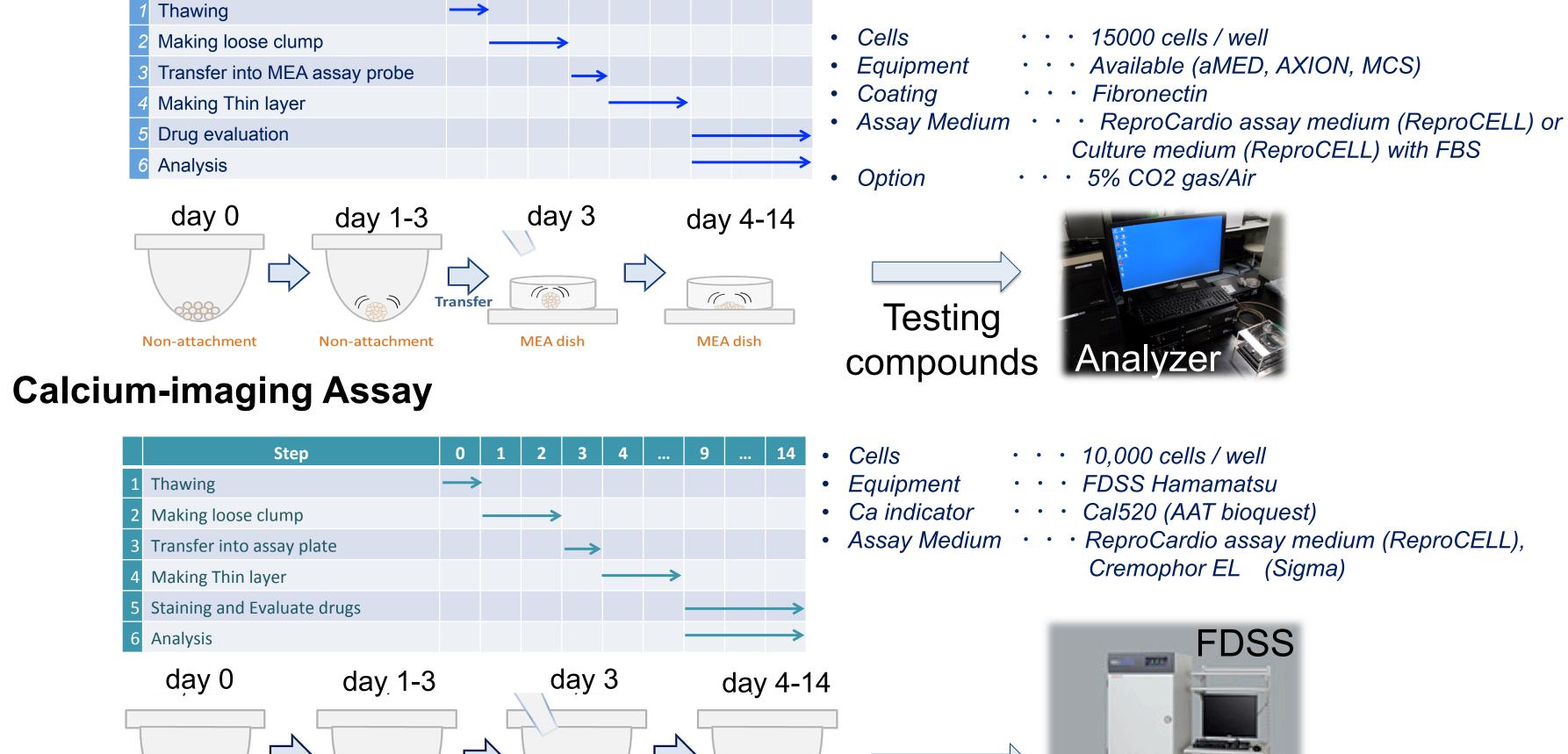
### ReproCardio2<sup>TM</sup> KIT (1 full kit for 96 well plate: ReproCELL # RCESD008)

- 1. 3.3×10<sup>5</sup> Single cells x 3 cryo-preserved vials
- 2. Empty Cryo-preserved Cell vial (dummy)
- 3. Maintenance culture medium (80mL) x 2
- 4. Coating solution (30mL) x 1
- 5. Low attachment plate for aggregated cells
- 6. Attachment plate for Thin-layer / Single cell

### Immunocytochemistry

- 1. Primary antibody: anti-Conexin 43, MYH6/7(Sigma); anti-MLC-2A/2V (Synaptic systems); anti-Troponin (A Bio-Rad company) and anti-Vimentin (Millipore).
- 2. Secondary antibody: Alexa Fluor 488 (Life Technologies)

### Electrophysiological assay



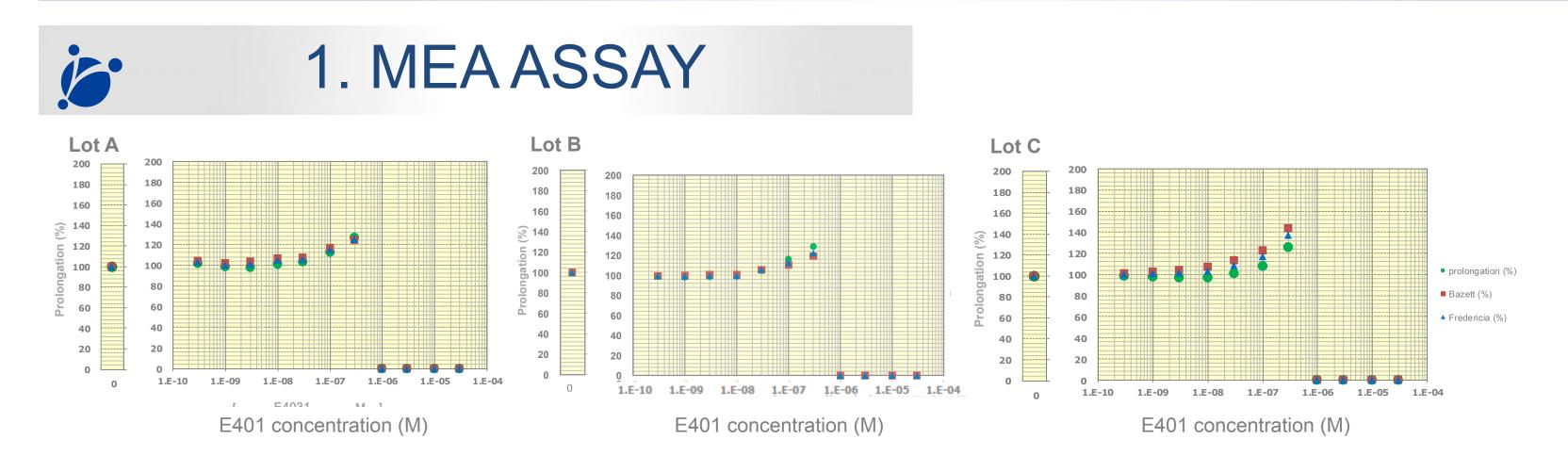
## Result

# Characteristic of ReproCardio2™

### Figure 1. ReproCardio2™ has various cardiac markers like human heart

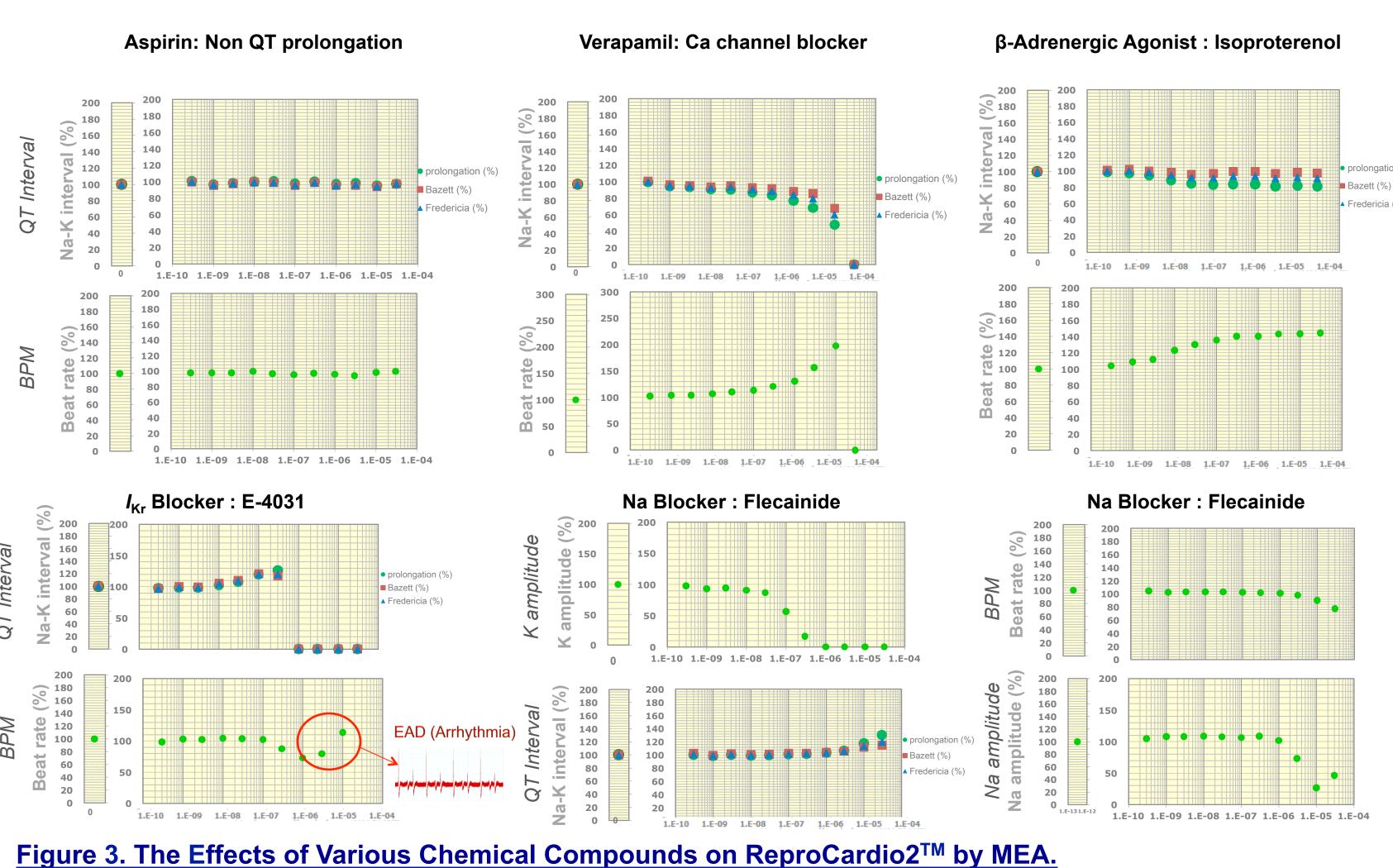
α-MHC and βMHC are the representative cardiac marker during the differentiation from iPS cells and expressed in heart. MLC-2A and MLC-2V are myosin specific marker for the atrium of the mammalian heart and for the ventricle of the mammalian heart, respectively. Cardiac troponin T (cTnT) is a specific marker in human heart and a thin filament protein which takes part in muscle contraction. Cx43 is conniving gap junction found in several organs and cell types, but is most often found in the heart. Those proteins were used as representative cardiac marker. Scale

## Electrophysiological Assay utilizing ReproCardio2<sup>TM</sup>

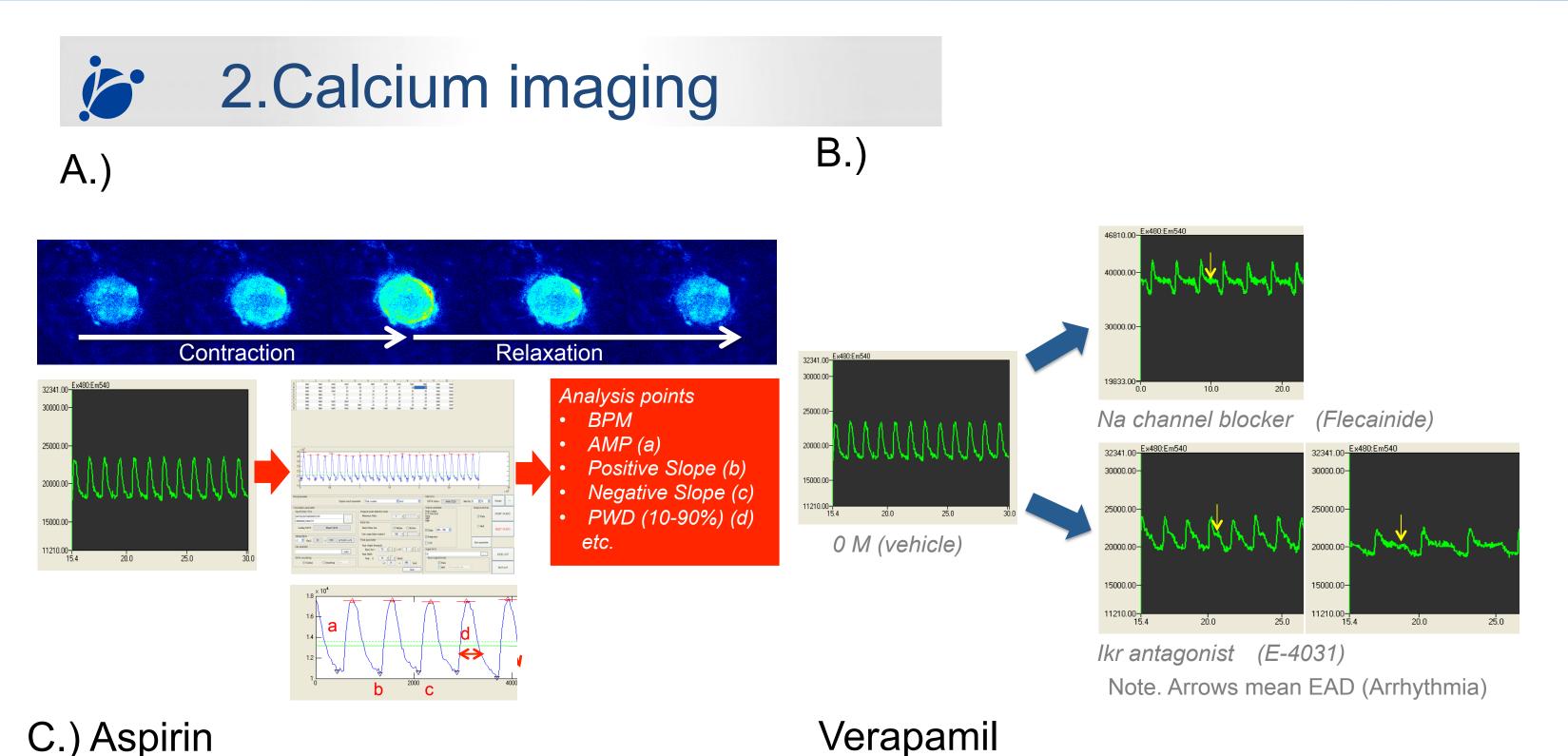


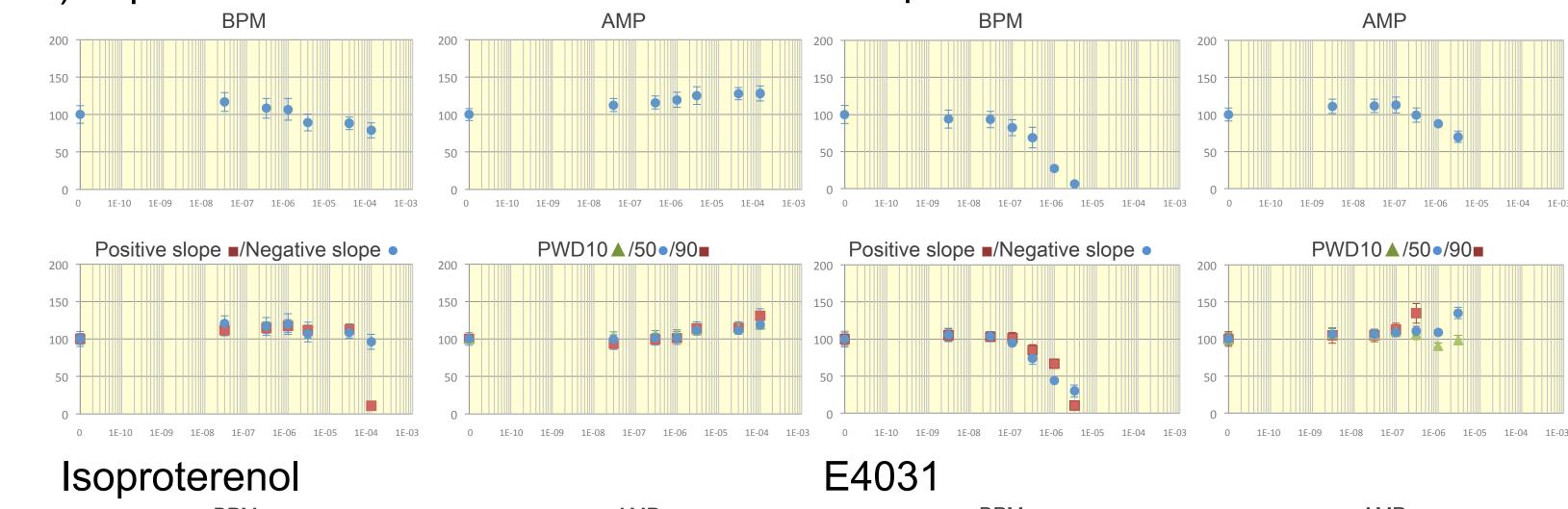
### Figure 2. Reproducibility of MEA assay: within the lot or between lot

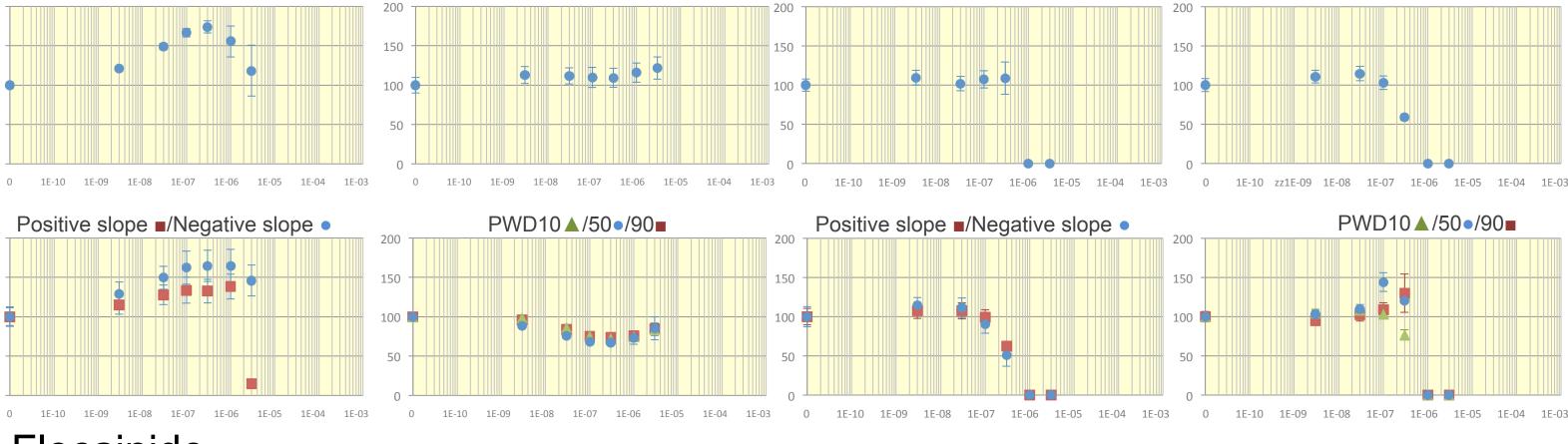
To prove there is no drastic difference within the cell lot, thin layers were made from cell clamps from the same vial of ReproCardio2<sup>TM</sup>. The different clamp-derived thin layers were exposed to various concentration of E4031 from 300 pM to 30 µM. The data obtained from each thin layers were plotted together, and it shows that E4031 has similar prolonging QT interval affect in all thin layer from the same cell lot. To prove cell lots has no drastic difference, we exposed various concentration of E4031 from 300 pM to 30µM to 3 different cell lots. The graphs showed that 3 different cell lots exhibit similar prolonging QT interval that is known for. This demonstrates that ReproCardio2<sup>TM</sup> has low production bias within lot as well as from lot to lot.

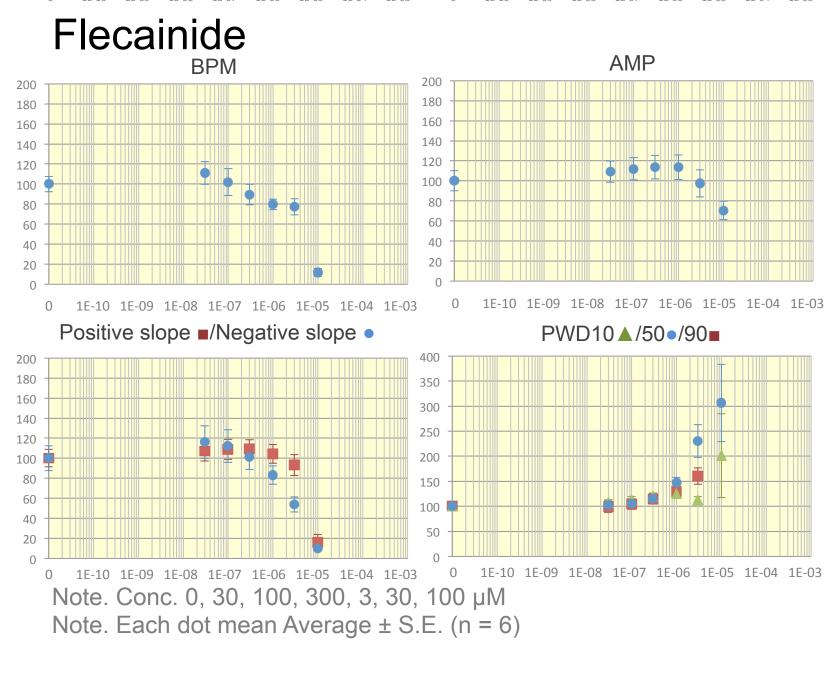


To see the affect of different drugs, thin layers were made from cell clamps. The thin layer ReproCardio2™ was exposed to various concentration of drugs from 300 pM to 30 µM. The data was obtained from MCS system, with 2 minutes of drug exposure and 2 minutes of recording.









### Figure 4. The Effects of Various Chemical Compounds on ReproCardio2™ by Calcium-imaging. A) The various parameters can be interpreted from Ca-

imaging. B) The change of graph after admitting drugs ReproCardio2<sup>TM</sup> was exposed to various concentration of drugs from 300pM-30µM. Ca signal from ReproCardio2™ can be detected for a hour. The data was obtained from 2 minutes of drug exposure and 2 minutes of recording.

## Conclusion & Discussion

Human iPS cell-derived cardiomyocytes ReproCardio2<sup>TM</sup> displays no differences between cell-lots, and exhibits constant affects of the drug screening even with different analyzing methods. The utility of ReproCardio2<sup>TM</sup> is also expandable. This demonstrated that ReproCardio2<sup>TM</sup> is suitable for the large-scale screening of chemical compounds.