

Frequently Asked Questions

1. How many cells are in each cluster?

Approximately 500-2000 cells/cluster

2. How fast are the cells beating and does this affect data acquisition and analysis?

Individual clusters of cells beat at different rates. This beating rate can affect interpretation of data. Various algorithms have been developed that can correct differences in data caused by beat rate and allow comparisons to be made between sample sets with different beat rates. In addition to utilizing these algorithms, as part of our quality control process, we use only cells with beating rate within a defined range (see below).

3. Does each cluster include a pacemaker cell?

We think so. Our myocyte clusters consist of many cells which beat in unison. If there is no pacemaker cell in each cluster, each cell would contract randomly. In this case, the contracting cells would arrest or the cluster would not produce an ECG-like signal.

4. How sensitive is the assay?

For the purpose of using these cells in a toxicity assay, both too high and too low sensitivity would cause the assay to lose utility. A good assay system produces results which correlate with the objective. In this case, it needs to accurately "call" negative compounds as negative and positive as positive with respect to the therapeutic dose of each compound. For the hERG assay, a compound is deemed safe if there is no effect on repolarization up to 10 fold therapeutic dose. QTempo consistently and accurately calls compounds correctly at doses below this ten-fold threshold. The range we have seen so far, which is highly dependent on the compound under study is 1-1000nm.

5. What are the QC controls to ensure reliability and consistency of data?

Please see separate file with detailed description.

6. In addition to QT prolongation, what other parameters can be identified?

The output of QTempo is an ECG-like waveform (an electro cardiomyograph or ECMG). The QTempo service is focused on measurement and analysis of QT/QTc. In addition to identifying QT prolongation, QTempo can identify QT shortening. Other parameters that can be measured include Na amplitude, K amplitude, width of QRS-like component, and Ca current. It should be noted that because each ion current is not clearly separated on ECMG (or ECG), the specific effect of a compound on each current is not always observed. If you are interested in these parameters or any other aspects of QTempo, please discuss this with us.

7. What is the difference between the electropotential measured in the QTempo assay and an ECG

The output for an ECG and an ECMG represent a composite derived from the action potential generated as a result of the coordinated flow of calcium, potassium and sodium ion channels across the cell membrane. The key differences between a clinical ECG and the ECMG waveform generated by QTempo are due to the (1) the source (2) the way the electrical activity is measured. In an ECG, the whole heart is measured from various points on the surface of the individual. In an ECMG a small group of cells are measured from a single measurement electrode (plus a reference electrode) which is in close proximity to the cells. Consequently, the waveform from an ECG is slightly more complex than for an ECMG. Equivalents of QRS and T regions are identifiable in an ECMG. However P and Q regions are not identifiable in an ECMG.

8. How long does it take to provide results?

Reports are typically sent by e-mail within a week of receipt of compound. We will provide an estimate at the time an order is placed.

9. What is the DMSO concentration used and what concentration can the cells tolerate?

We recommend 0.3% or lower concentration of DMSO, if 1% DMSO or higher is required, we would need to perform a vehicle assay as control using the higher concentration of DMSO before the actual assay.

10. ReproCELL screens cardiomyocytes for standard function prior to use in an assay. What is the failure rate of our cell QC criteria?

Currently, it is around 50% and falling. When cells are dissected from a plate, based on gross characteristics, it is possible to predict the quality of cells. Good base functionality is not only associated with the quality of the cells but also with the quality of contact between cells and electrode. We are developing tools to reduce this area of variability.

11. Do the cells always take 12 days to start beating? What is the range?

Around 12 days is typical, but cells commence beating anywhere between 10~14-day post induction of differentiation.

12. What are slow and fast beat rates where we see differential effects, for example, with Chromanol 293B?

We performed the assay on cells beating at 10 bpm and 200 bpm. We are planning to perform the assay at a range of beats to find the beat rate at which QT prolongation is initially observed

13. Can serum be used in the assay?

We have developed serum free medium to remove effects that may be caused by sequestration of a compound by proteins. Serum may be included in the assay if required.

14. Atrial vs ventricular cells. What is the representation?

This assay is performed using cells that have been differentiated for 2~4 weeks. Our cell's functionality for QTempo assay is good but actual heart differentiation in human is much longer. The functional and genetic data we have so far lead us to think our cells may be characterize as premature ventricular like cells.

15. What is the assay volume?

Currently 1~1.5ml/single-assay dish. This will be reduced in future.

16. Do we know if compounds are getting inside the cell?

If a researcher is interested in whether or not their compounds are getting inside the cells we will send cell lysate prepared from treated with compound and outside washed cells. An additional fee will be charged.

17. What is the effect of pH?

We perform QTempo in the range pH 7.2~7.6 High and low pH destroy assay functionality.

18. How do we compensate for plastic binding properties of particular drugs?

We are aware of this issue – which affects any assay system using plastics. We typically test compounds to high concentration so can expect that at least some compound will reach the cells at high concentrations.

19. Sodium blockers tend to be more effective at prolonging QT interval at higher beat rates. Have ReproCell looked at this?

Not yet. We are planning to test this.

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