

# Assay Report

Customer's Test ID:  
RC Test ID:  
PO#:

Service name: **QTempo monkey ES**:QT prolongation assay by monkey ES cell derived beating cardiomyocyte  
Product code: RCESD001

## Customer Information

Name:            Division:            Organization:

## Test sample information

Sample ID(name):compound 1, compound 2

Dose:

compound 1:0pM, 300pM, 1nM, 3nM, 10nM, 30nM, 100nM, 300nM, 1uM, 3uM,  
10uM, 30uM

compound 2:0pM, 300pM, 1nM, 3nM, 10nM, 30nM, 100nM, 300nM, 1uM, 3uM,  
10uM, 30uM  
(N=1)

## Schedule

2009/2/23 ~ 2009/3/6

## Conducted by: ReproCELL Inc.

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## Responsible person:            Yasuyuki Asai, Ph. D.

Chief Technology Officer

signature/date\_\_\_\_\_ / \_\_\_\_\_.

## Materials & Methods

1-a. **QC definition** Size of beating cardiomyocyte clump:

Each cell clump was 100-300  $\mu\text{m}$  diameter (thickness not defined).

Actual size of each clump to be described in the report.

1-b. **QC definition** Magnitude/amplitude of voltage from beating clumps

• Na amplitude: 40  $\mu\text{V}$  or larger (between first positive and negative peak)

• K amplitude: 2  $\mu\text{V}$  or larger (between first positive and negative peak) and opposite polarity of Na wave. Beginning of K-wave is within 600 msec. from beginning of Na-wave with no treatment.

Actual amplitude will be described in the report.

1-c. **QC definition** Beat rate

40~120 beats/ minute (Average from 3-minute measurement with no treatment)

Actual beat rate will be described in the report.

2. Device/Instrument

• MEA system with temperature controller from multi channel systems GmbH (Germany)

• 30/200 electrode dish from multi channel systems GmbH (Germany)

• Software: "QT screen" from multi channel systems GmbH (Germany)

3. Procedure/method

All procedures are carried out at 37 degree C.

1) 3-minute pre-acquisition from beating clump without treatment.

2) The clump passing QC definition is confirmed by analysis of initial data.

3) Each concentration of test compound is added to beating clump every 4 minutes. (Compound treatment is accumulatively) The first 2-minute (0 to 2 min.) treatment is for conditioning, and the second 2-minute (2 to 4 min.) treatment is for "Detection".

• Dose (final concentration):

compound 1: 0pM,

300pM, 1nM, 3nM, 10nM, 30nM, 100nM, 300nM, 1 $\mu\text{M}$ , 3 $\mu\text{M}$ , 10 $\mu\text{M}$ , 30 $\mu\text{M}$

compound 2: 0pM,

300pM, 1nM, 3nM, 10nM, 30nM, 100nM, 300nM, 1 $\mu\text{M}$ , 3 $\mu\text{M}$ , 10 $\mu\text{M}$ , 30 $\mu\text{M}$

n=1

\* Toxic compound may arrest the beating of clump. Once beating is arrested, the experiment is terminated and any effect in higher concentration is not observed.

• solvent/vehicle: dimethyl sulfoxide (DMSO)

## Results

1-a. Size of beating cardiomyocyte clump:

**For compound 1: 300 um diameter**

**For compound 2: 300 um diameter**

1-b. QC definition Magnitude/amplitude of voltage from beating clump

•Na amplitude: 40 uV or larger (between first positive and negative peak)

•K amplitude: 2 uV or larger (between first positive and negative peak) and opposite polarity of Na wave. Beginning of K-wave is within 600 msec. from beginning of Na-wave with no treatment.

**Na amplitude of beating for compound 1: 100 uV,**

**K amplitude of beating for compound 1: 2.5 uV**

**Na amplitude of beating for compound 2: 60 uV,**

**K amplitude of beating for compound 2: 3.0 uV**

1-c. QC definition Beat rate

40~120 beats/ minute (Average from 3-minute measurement with no treatment)

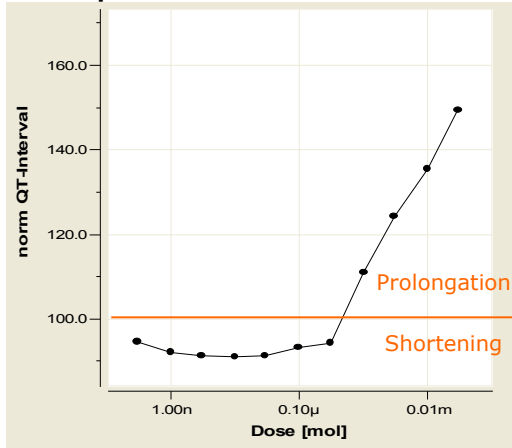
Actual beat rate:

**Beat rate of cell clump for compound 1: 59 bpm**

**Beat rate of cell clump for compound 2: 40 bpm**

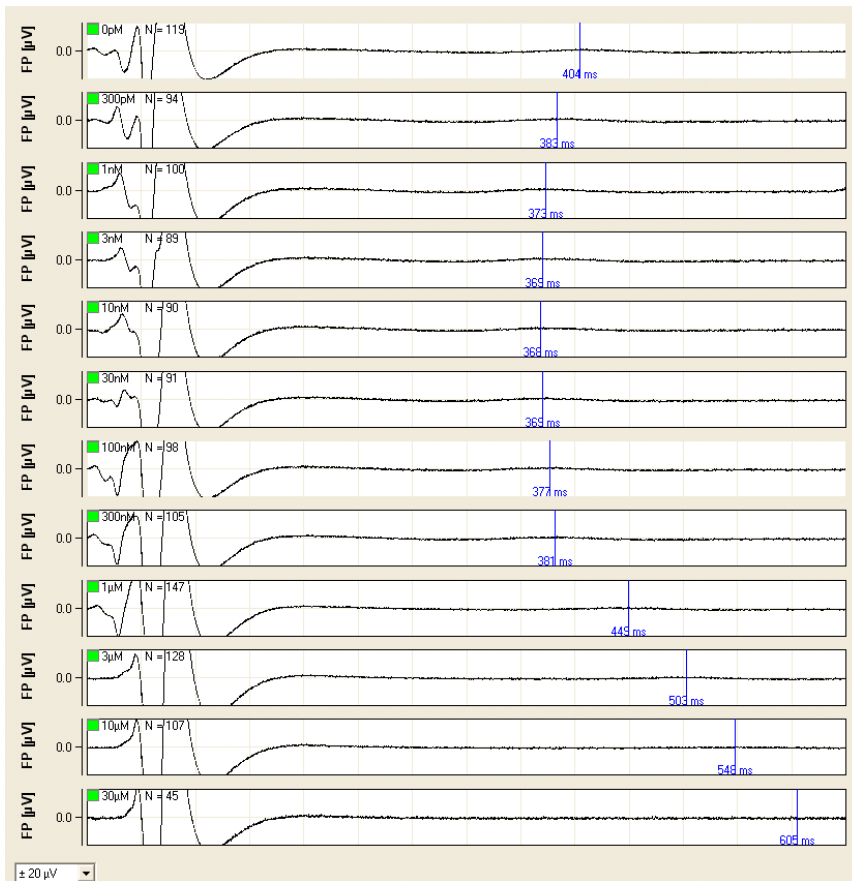
Results –continued-

compound 1



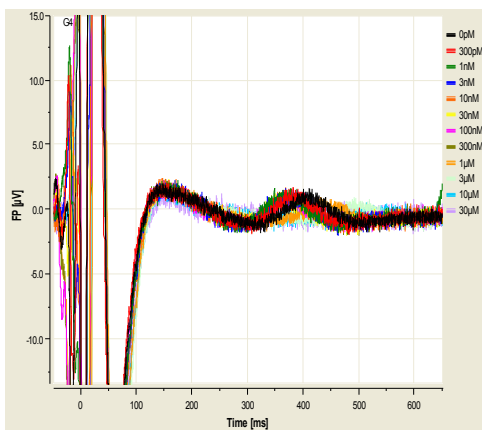
**Dose dependent curve of compound 2 on Na-K interval.**

QT (Na-K interval) prolongation was observed.



**Waveform of each concentration of compound 1.**

K-peak (Blue line) was shifted to late position.

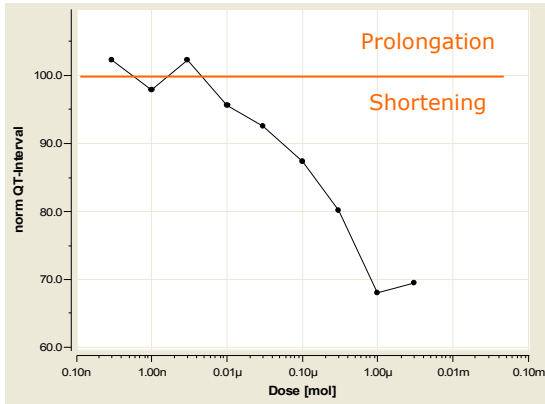


**Overlaid waveform of compound 1 treatment.**

In addition to K-peak shift, K-peak height was decreased.

Results –continued-

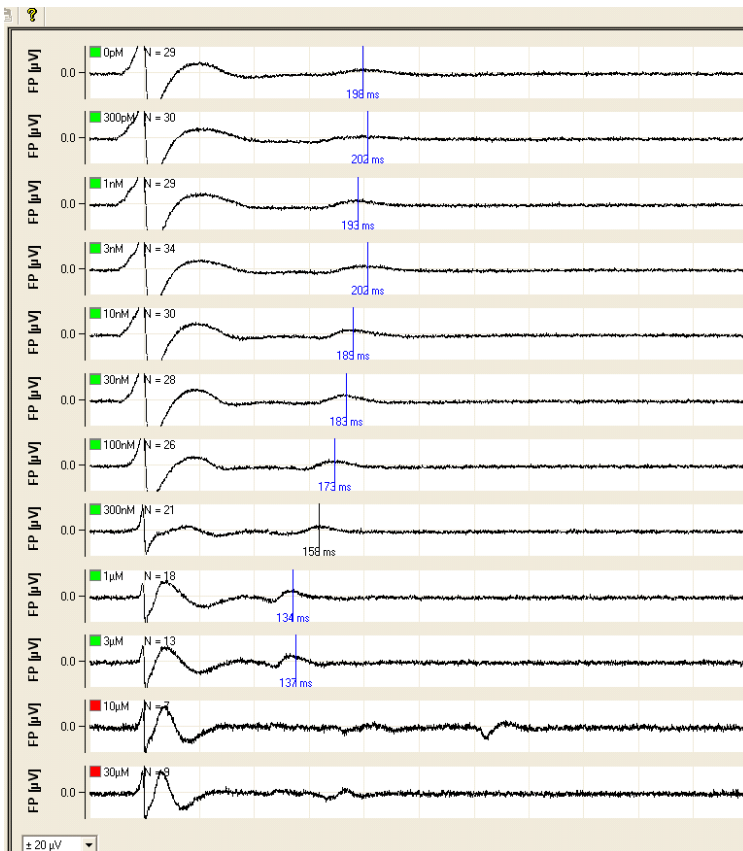
compound 2



**Dose dependent curve of compound 2 on Na-K interval.**

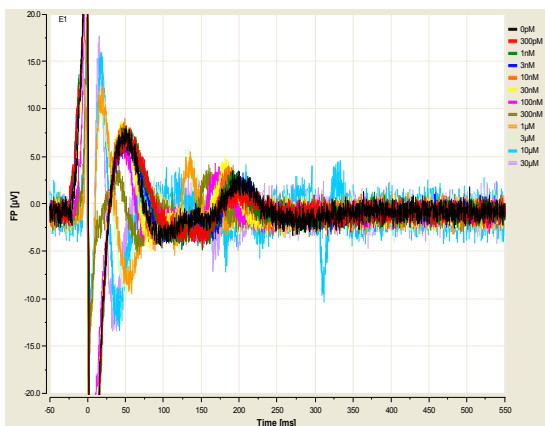
QT (Na-K interval) prolongation was not observed.

QT (Na-K interval) shortening observed.



**Waveform of each concentration of compound 2.**

K-peak was shifted to earlier position. The highest and second highest concentration of compound 2 affected beating itself, K-peak was not detectable.



**Overlaid waveform of compound 2 treatment.**

In addition to QT shortening Ca current blockade potential was observed.