

Protocol

Service Name: QTempo assay service

Service Code:

Experimental ID:

Customer Information: Name:

E-mail:

E-mail capacity: —

Service by ReproCELL, Inc.

URL: www.reprocell.com

Responsible person:

Signature: _____ Date/ _____

Schedule:

- | | | |
|--------------------------------|------------|--------------|
| 1. Test compounds shipped by | on | 2015/xx/xx |
| <hr/> | | |
| 2. Assay at ReproCELL during | 2015/xx/xx | ~ 2015/xx/xx |
| <hr/> | | |
| 3. Quick report from ReproCELL | on | 2015/xx/xx |
| <hr/> | | |
| 4. Final report from ReproCELL | on | 2015/xx/xx |
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Materials and Methods

1. Cells: Cardiomyocyte derived from human iPS cell

- 1-1. Stem cell line name: RCHIPC004: derived from Human cardiac myocyte
1-2. Reprogrammed by RCHIPC004, sox2, oct3/4, klf4, c-myc with retrovirus

2. Assay medium: QTempo Assay medium Cat.No.RCESD007

3. Electrode plate/dish name: MEA dish 200/30iR-Ti-gr (Multi channel systems)

4. Instrument: MEA system with temperature controller (Multi channel systems)

5. Software: LabChart v7

6. Test compound(s)

total ____ compounds

Procedure

A. Quality control (QC):

Human iPS derived cardiomyocyte passed items as described below can be applied to assay.

As cardiomyocyte consisting from cells have passed quality control criteria explained below, the results from different cardiomyocyte can be compared to each other regardless of the difference of the waveforms.

A-1. Magnitude/amplitude of voltage from beating thin-layers:

- Na amplitude: 40 μ V or larger (between first positive and negative peak)
 - K amplitude: 2 μ 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.

A-2. Beat rate: 30~ beats/ minute (Average from 3-minute measurement with no treatment)

Actual beat rate will be described in the report.

B. Assay

All procedures including QC process are carried out at 37 degree C.

B-1. 3-minute pre-acquisition from beating thin-layer without treatment for A-1 and A-2.

B-2. The cardiomyocyte passing QC definition is confirmed by analysis of initial data from B-1.

B-3. Each concentration of test compound is added to beating thin-layer 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".

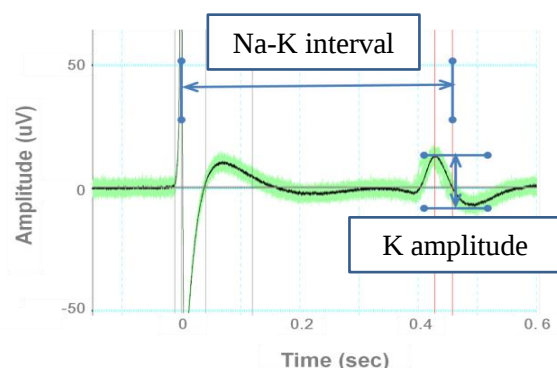
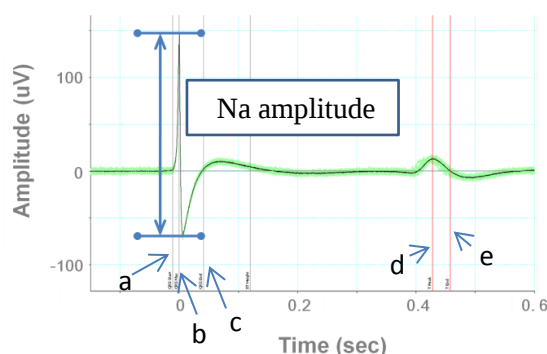
- Sample ID (name) : as written in "Materials and Methods"
- Assay concentration (final concentration of test compound)
: Reference (Shinpping list from -)

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

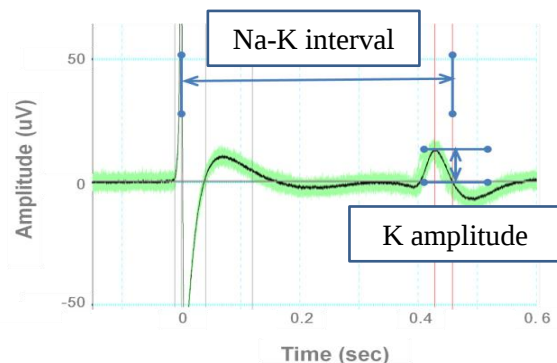
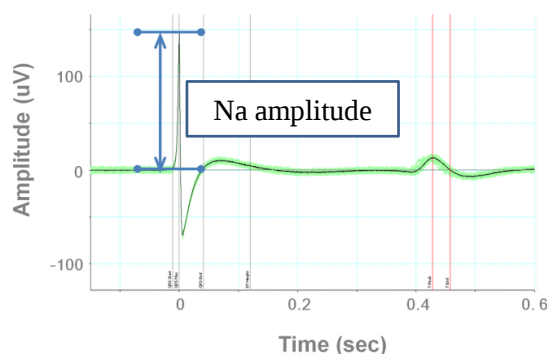
- number of experiment : n = 3 (E4031,)
- Compound Treatment Medium : QTempo Assay Medium (Cat.No.RCESD007)

C. Detection points

C-1. QC



C-2. With compound



a: Na amplitude start b: Na amplitude peak c: Na amplitude end d: K amplitude peak e: K amplitude end

Note: Na-K interval : Time until K amplitude end from Na amplitude start. (sec)

Note: Line colors are chosen just for the purpose of visibility and do not have any particular scientific meanings about the assay :

D. Results

Na amplitude (μV)

K amplitude (μV)

Na-K interval (sec)

Bazzet : $\text{Na-K interval (sec)} / \sqrt{\text{ISI}}$

Fridericia : $\text{Na-K interval (sec)} / \sqrt[3]{\text{ISI}}$

Inter spike interval (ISI) : Time (sec) between two adjoining Na-peaks

Wave form (green line) : Overlayed all waves of Na-K interval in the assay term (※Black line : Averaged wave)

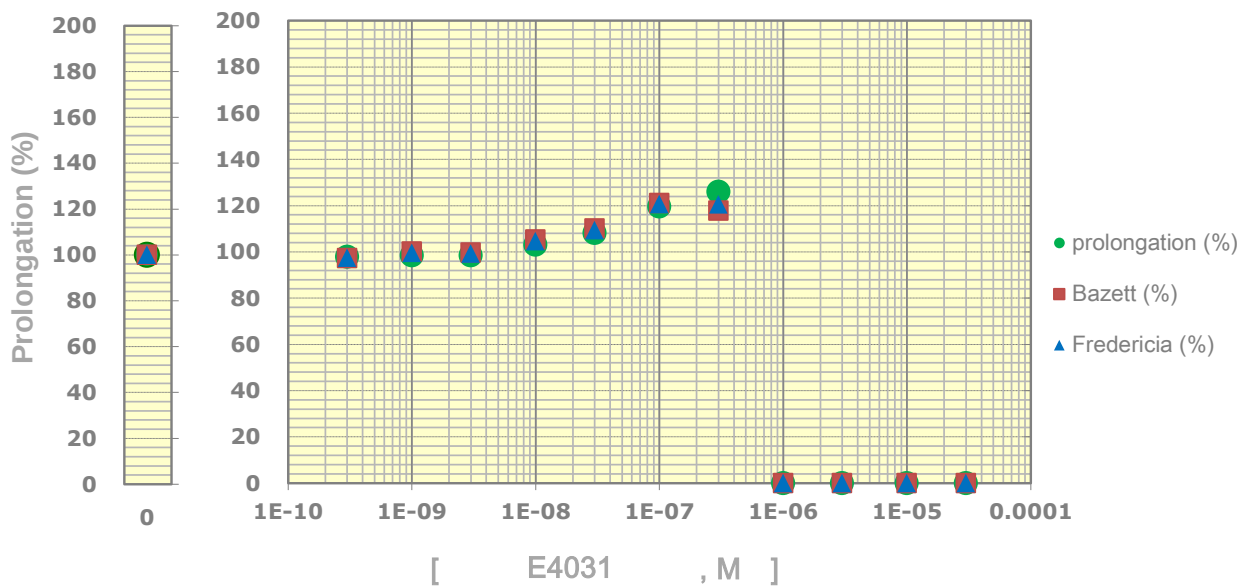
No.	Compound name	
1		16
2		25
3		34
4		43
5		52
6		61
7		70
8		79
9		85
10		91
11		97

Results

【Test compound name】
E4031

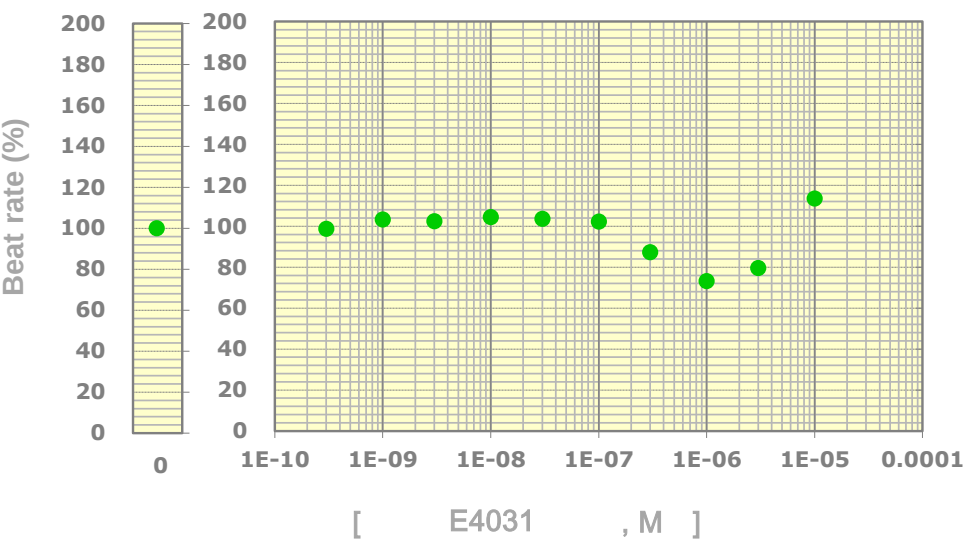
【QC Results】
Beat rate 56 bpm
Clump size 200 um x 200 um
Na amplitude 70 uV
K amplitude 3.3 uV
Na-K interval 0.466 sec.

1. Na-K interval



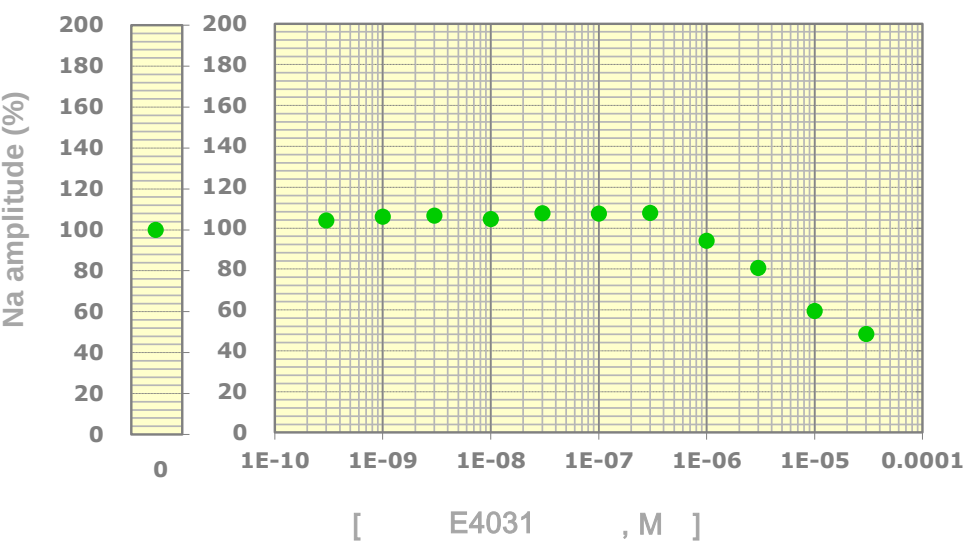
	0 M	300 pM	1 nM	3 nM	10 nM	30 nM	100 nM	300 nM	1 uM	3 uM	10 uM	30 uM
ISI	1.14	1.16	1.11	1.12	1.10	1.10	1.12	1.31	1.56	1.44	1.01	0.56
Na-K Interval (sec)	0.465	0.455	0.457	0.458	0.479	0.503	0.556	0.585	0.000	0.000		
prolongation(%)	100	98	98	98	103	108	120	126	0	0	#####	#####
Bazett	0.434	0.422	0.435	0.433	0.458	0.478	0.526	0.512	0.000	0.000	#####	#####
prolongation(%)	100	97	100	100	105	110	121	118	0	0	#####	#####
Fredericia	0.444	0.433	0.442	0.441	0.465	0.486	0.536	0.536	0.000	0.000	#####	#####
prolongation(%)	100	97	100	99	105	109	121	121	0	0	#####	#####

2. Beat rate



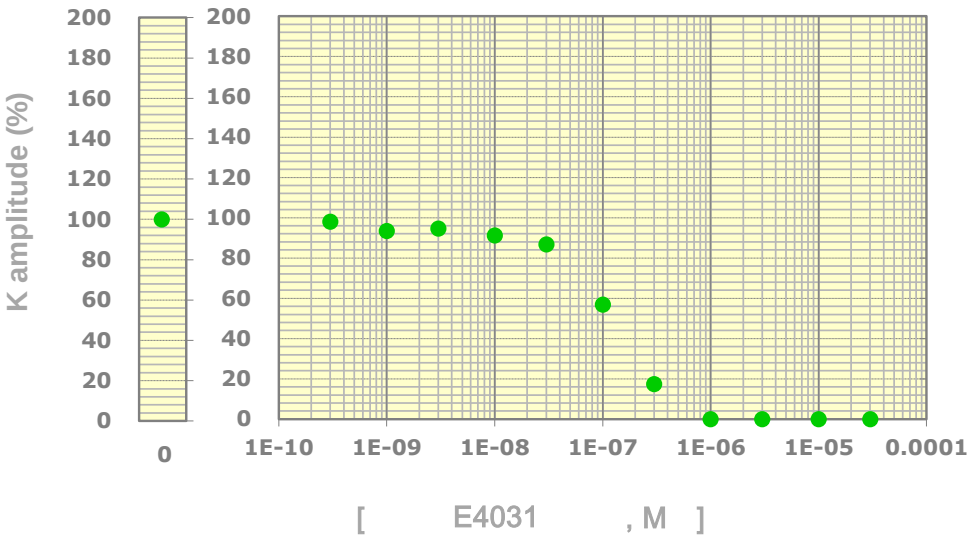
	0 M	300 pM	1 nM	3 nM	10 nM	30 nM	100 nM	300 nM	1 uM	3 uM	10 uM	30 uM
Beat rate (bpm)	52	52	54	54	55	54	54	46	38	42	60	107
%	100	99	103	102	104	104	102	87	73	80	114	203

3. Na amplitude



	0 M	300 pM	1 nM	3 nM	10 nM	30 nM	100 nM	300 nM	1 uM	3 uM	10 uM	30 uM
Na amplitude (uV)	64	66	67	67	66	68	68	68	60	51	38	31
%	100	103	105	105	103	106	106	106	94	80	59	48

4. K amplitude



	0 M	300 pM	1 nM	3 nM	10 nM	30 nM	100 nM	300 nM	1 uM	3 uM	10 uM	30 uM
K amplitude (uV)	2.6	2.5	2.4	2.5	2.4	2.3	1.5	0.4	0.0	0.0	0.0	0.0
%	100	98	93	95	91	87	57	17	0	0	0	0