

# High-throughput assessment of drug effects on calcium transients in human induced pluripotent stem cell (hiPSC) derived cardiomyocytes

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## Abstract

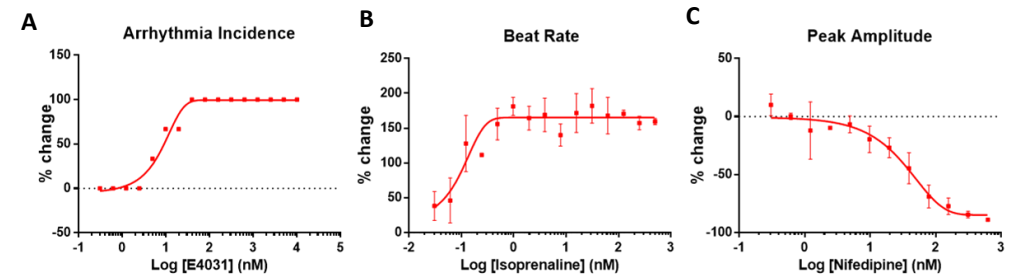
There is a pressing need for predictive *in vitro* assays suitable for high-throughput screening (HTS) to detect cardioactive effects of compounds early in the drug discovery process. Human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes are a relevant *in vitro* model for this purpose.

We have recently developed fully functional hiPSC-derived ventricular cardiomyocytes (Pluricyte<sup>®</sup> Cardiomyocytes) which exhibit a relatively high level of maturity. This was demonstrated by an increased contraction profile, a highly organized sarcomere organization, as well as improved electrophysiological properties (negative resting membrane potential, well defined action potential plateau and rapid depolarization) as depicted in **Figure 1** [1] and **Figure 5**.

To assess acute cardiotoxicity, we developed a high-throughput drug screening assay by combining the FLIPR Tetra<sup>®</sup> (Molecular Devices) screening platform with Pluricyte<sup>®</sup> Cardiomyocytes and a fluorescent calcium dye. After optimization of the assay for application with 384-well plates, we investigated the impact of different cardioactive compounds (e.g. hERG-channel blockers, calcium channel agonists/antagonists and  $\beta$ -adrenergic agonists) on the calcium transients in Pluricyte<sup>®</sup> Cardiomyocytes. The results obtained with the FLIPR Tetra<sup>®</sup> screening platform were validated by comparing the calcium transient data to the data obtained by medium-throughput multielectrode array (MEA)/impedance assays.

We conclude that Pluricyte<sup>®</sup> Cardiomyocytes in combination with calcium flux assays on a high-throughput drug screening system provide a highly relevant *in vitro* assay to study cardiac safety of pharmaceuticals.

## Quantification of drug-induced alterations of calcium transients in Pluricyte<sup>®</sup> Cardiomyocytes measured by the FLIPR Tetra<sup>®</sup> platform

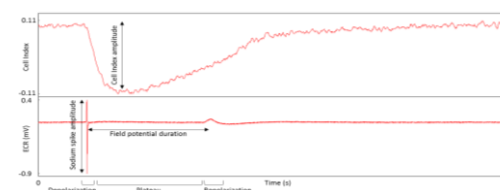


**Figure 4. Concentration-response curves of cardioactive compounds determined by measuring very acute alterations in calcium transients of Pluricyte<sup>®</sup> Cardiomyocytes.**

- (A) Blocking of the hERG channel with increasing concentrations of E4031 resulted in arrhythmic-like events.  
 (B) The  $\beta$ -adrenergic receptor agonist isoprenaline induced an increase in beat rate.  
 (C) Increased concentration of L-type calcium channel blocker nifedipine reduced the calcium transient peak amplitude, eventually leading to complete diminishing of the signal.

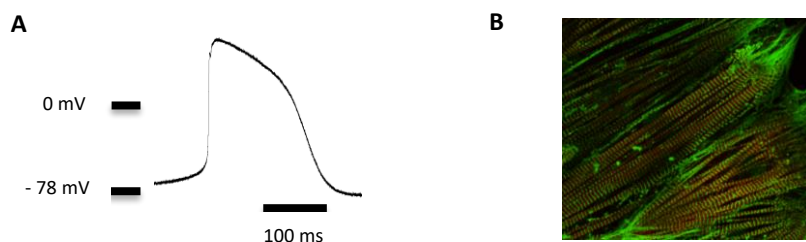
## Validation of calcium transient data:

### Compound effects on electrophysiology and contraction of Pluricyte<sup>®</sup> Cardiomyocytes



**Figure 5. A typical waveform of a Pluricyte<sup>®</sup> Cardiomyocyte monolayer acquired through MEA analysis.** Cell Index (i.e. impedance, a surrogate marker for contractility) signal (top) and ECR (i.e. field potential) signal (bottom) obtained using the xCelligence<sup>®</sup> RTCA CardioECR instrument (ACEA Biosciences). The field potential shows a robust sodium spike amplitude and a well-pronounced repolarization peak.

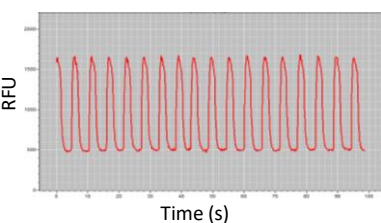
## Human Pluricyte<sup>®</sup> Cardiomyocytes are fully functional and show a relatively high level of maturity



**Figure 1. Characteristics of Pluricyte<sup>®</sup> Cardiomyocytes.**

- (A) A typical action potential of Pluricyte<sup>®</sup> Cardiomyocytes, demonstrating a low resting membrane potential and fast upstroke velocity.  
 (B) Pluricyte<sup>®</sup> Cardiomyocytes exhibit a high degree of ultra-structural organization of the sarcomeres as determined by immunofluorescence (Green: alpha actinin; Red: Myosin heavy chain 7).

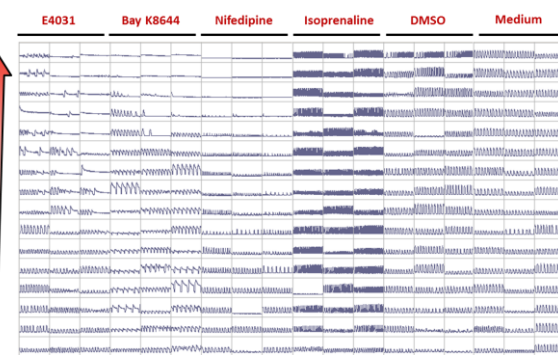
## Screening of drug-induced alterations of calcium transients in Pluricyte<sup>®</sup> Cardiomyocytes using the FLIPR Tetra<sup>®</sup> platform



**Figure 2. A representative calcium transient fluorescent signal of Pluricyte<sup>®</sup> Cardiomyocytes.** Calcium transient data are obtained from Pluricyte<sup>®</sup> Cardiomyocytes treated with 0.25% DMSO (negative control) using the FLIPR Tetra<sup>®</sup> platform. The FLIPR Calcium 6 Assay Kit (Molecular Devices) was used to detect calcium-transients.

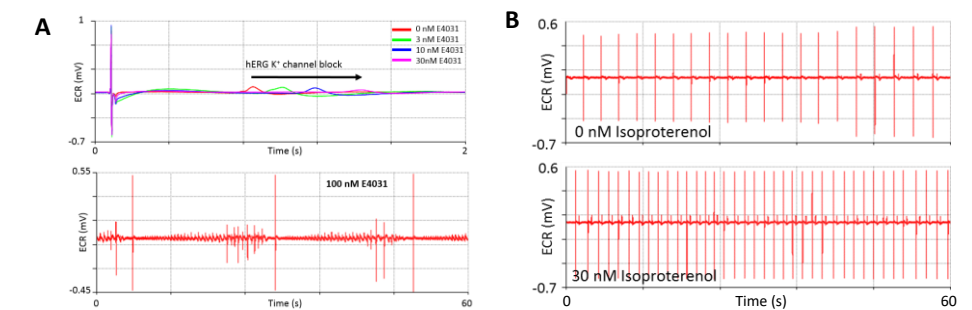
Row	[E4031] nM	[Bay K8644] $\mu$ M	[Nifedipine] nM	[Isoprenaline] nM	[DMSO] %
A	10000	100	10000	1000	1
B	5000	50	5000	500	0.5
C	2500	25	2500	250	0.25
D	1250	12.5	1250	125	0.125
E	625	6.25	625	62.5	0.063
F	313	3.13	313	31.25	0.031
G	156	1.56	156	15.63	0.016
H	78	0.78	78	7.81	0.008
I	39	0.39	39	3.91	0.004
J	20	0.20	20	1.95	0.002
K	10	0.10	10	0.98	9.77E-04
L	4.9	0.05	4.9	0.49	4.88E-04
M	2.4	0.02	2.4	0.24	2.44E-04
N	1.2	0.01	1.2	0.12	1.22E-04
O	0.6	0.006	0.6	0.06	6.10E-05
P	0.3	0.003	0.3	0.03	3.05E-05

**Table 1. Experimental set-up.** The table shows the final test concentrations of different cardioactive compounds with increasing concentrations from bottom to top. Effects of these compounds on calcium transients in Pluricyte<sup>®</sup> Cardiomyocytes are presented in **Figure 3**.



**Figure 3. Screenshot of acute compound effects on the calcium transient signals of Pluricyte<sup>®</sup> Cardiomyocytes.**

The measurement was performed directly after compound addition. The screenshot shows a 60 seconds time scale. We could observe:  
**Proarrhythmic effects:** Increasing concentrations of E4031 (hERG channel blocker) resulted in arrhythmia-like events.  
**Positive inotropic effects:** Bay K8644 (calcium channel agonist) caused increased peak amplitudes and increased peak widths.  
**Negative inotropic effects:** Nifedipine (calcium channel blocker) reduced peak amplitudes.  
**Positive chronotropic effects:** Isoprenaline ( $\beta$ -adrenergic receptor agonist) caused an increase in beat rate.



**Figure 6. Pharmacological response of Pluricyte<sup>®</sup> Cardiomyocytes to cardioactive compounds on field potential and impedance using the xCelligence<sup>®</sup> RTCA CardioECR instrument.**

- (A) E4031, a hERG channel blocker, induced a concentration-dependent increase of the field potential duration and flattening of the repolarization peak. Arrhythmic-like events were observed at  $\geq 100$  nM.  
 (B) Isoproterenol, a  $\beta$ -adrenergic receptor agonist, induced an increase in beat rate (+50% change in beat rate at 30 nM compared to baseline).  
 (C) Nifedipine, an L-type calcium channel blocker, induced a decrease in field potential duration, as well as a decrease in impedance peak amplitudes (-35% change in peak amplitude at 300 nM compared to baseline).

## Concluding Remarks

- Pluricyte<sup>®</sup> Cardiomyocytes cultured in Pluricyte<sup>®</sup> Cardiomyocyte Medium show a ventricular, relatively mature phenotype.
- Pluricyte<sup>®</sup> Cardiomyocytes can be efficiently used to detect **positive and negative chronotropic and inotropic effects, as well as proarrhythmic effects of test compounds on cardiomyocytes in a high-throughput calcium flux assay.**
- The calcium transient data showed a profile that was in line with the compound effects observed in MEA and impedance assays.
- Calcium-transient analysis in Pluricyte<sup>®</sup> Cardiomyocytes using the FLIPR Tetra<sup>®</sup> platform provides a robust high-throughput screening method, which can be used to study cardiac safety of pharmaceuticals and which supports the use of these assays for efficacy screenings.

## References

[1] Ribeiro MC et al., Functional maturation of human pluripotent stem cell derived cardiomyocytes *in vitro* - correlation between contraction force and electrophysiology. *Biomaterials* (2015) 51:138-50.

## Acknowledgments

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