

eBook contents

Cardiomyocytes

In vitro cardiotoxicity assessment of environmental chemicals using an organotypic human induced pluripotent stem cell-derived models.....2

Assessment of drug effects on cardiomyocyte physiology using human iPSC-derived cardiac spheroids.....3

Compound effects upon calcium transients in beating Ncardia Cor.4U human iPSC-derived cardiomyocytes.....4

High throughput cardiotoxicity assays using stem cell-derived cardiomyocytes.....5

In vitro assessment of drug effects on human iPSC-derived cardiac spheroid cultures.....6

Neurons

Functional and mechanistic neurotoxicity profiling using human iPSC-derived neural 3D cultures.....7

Calcium flux assay for *in vitro* neurotoxicity studies and drug screening.....8

About the instruments9

Phenotypic screening with iPSC-derived cardiomyocytes and neurons

There is a critical need for more relevant models to identify therapeutic effects and adverse responses to compounds earlier in the drug discovery process. Profiling the effects of environmental hazards in a higher throughput fashion is extremely important. The development of human induced pluripotent stem cells (iPSC) has made it possible to develop new higher throughput screening assays to study compound effects upon iPSC cardiomyocytes and neuronal cultures.

Cytotoxicity and mitochondrial integrity studies using the ImageXpress® Micro Confocal High-Content Imaging System with both 2D and spheroid cultures provide an accurate platform for data analysis. Spontaneous calcium oscillation acquired with FLIPR® Calcium 6 Assay Kits and the FLIPR® High-Throughput Cellular Screening System from both iPSC cardiomyocytes and neuronal cultures can be paired with cytotoxicity and mitochondrial integrity data from the ImageXpress Micro Confocal system for comprehensive phenotypic analysis and bioactivity profiling of both pharmaceutical and environmental compounds.

In this eBook, we use both imaging and calcium oscillation analysis to develop profiles of compounds in iPSC-derived cardiomyocytes such as hERG blockers, β -adrenergic agonists, and environmental toxins. iPSC-derived neuronal cultures were evaluated with neuromodulators as well as environmental toxins.



For more information, visit
www.moleculardevices.com



In vitro cardiotoxicity assessment of environmental chemicals using an organotypic human induced pluripotent stem cell-derived model

READ PAPER ►

- Fast kinetic fluorescence imaging was used, which monitored changes in intracellular Ca^{2+} using a calcium sensitive dye to screen a library of environmental chemicals and drugs for their ability to alter calcium oscillation rates and patterns in human iPSC-derived cardiomyocytes.
- High content imaging was also used to monitor the effect of the compounds on mitochondrial cytotoxicity with the same cell model.
- Assay was optimized for high throughput screening by using multi-parameter analysis outputs such as beating rate, peak frequency and width, amplitude, or wave form irregularities.

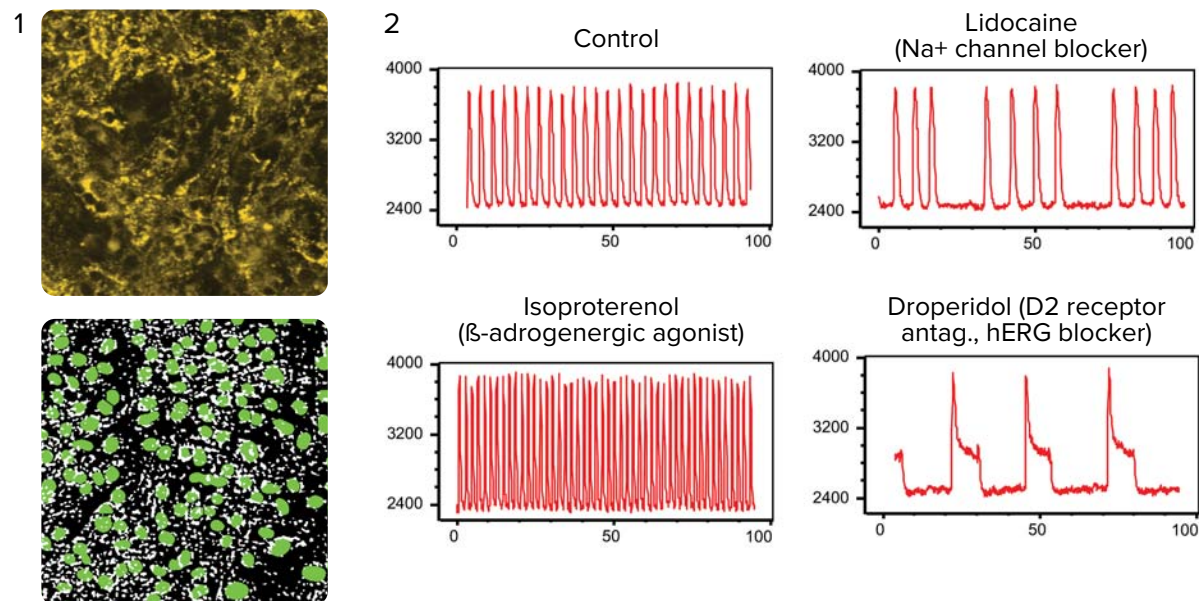


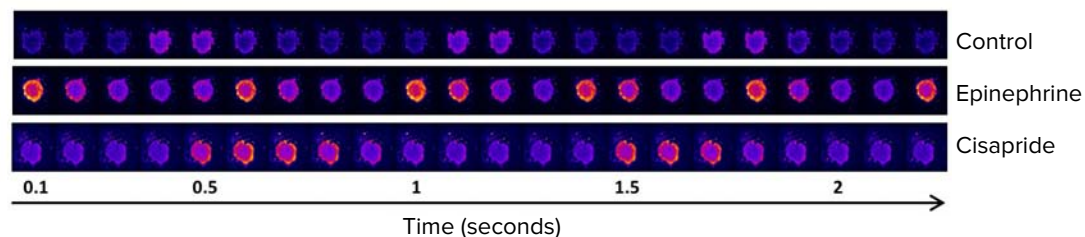
Figure 1. Images of cardiomyocytes from Mitochondria Toxicity assay. Top: Cells stained with JC-10 dye. **Bottom:** Granularity analysis results. Green masks indicate nuclei; White dots indicate mitochondria stain. **Figure 2. Representative calcium-flux signal traces for different chemical classes.**



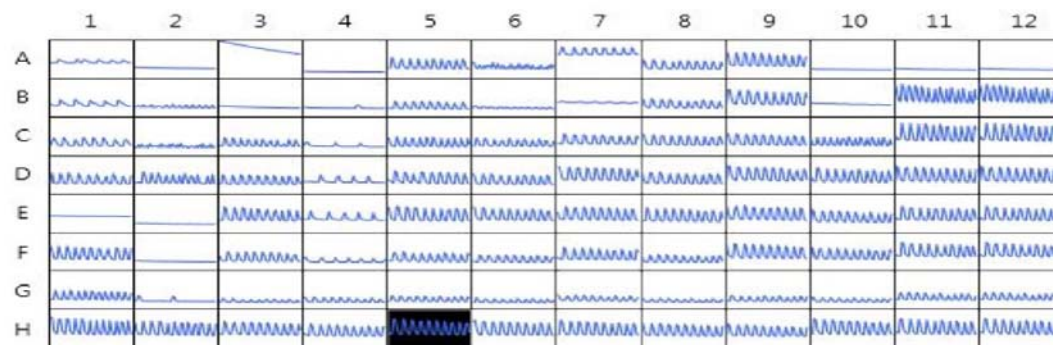
Assessment of drug effects on cardiomyocyte physiology using human iPSC-derived cardiac spheroids

READ APPLICATION NOTE ►

- Acquire timelapse images and analyze cardiac spheroid beating patterns using high-content imaging
- Obtain simultaneous kinetic reading of calcium flux across all the wells using FLIPR High-Throughput Cellular Screening System
- Measure the impact of different compounds on cardiac spheroid viability



Time-lapse image series of contracting iPSC-derived cardiomyocyte spheroids loaded with a Ca^{2+} sensitive dye. Each image was taken at 100 millisecond intervals. Yellow/red (false color scale) indicates a high Ca^{2+} concentration and a state of contraction.



Kinetic fluorescent intensities of compound-treated cardiac spheroids. Image data was imported into the SoftMax® Pro 7 Software for analysis of beating rates, amplitudes, peak width, and other readouts. Shown here is a plate view of the time-lapse intensity plots.



- Evaluate compound toxicity and efficacy earlier in the drug discovery process
- Analyze cardiotoxicity profiles in a biorelevant system
- Scale assay size to meet throughput requirements

Compound effects upon calcium transients in beating Ncardia Cor.4U human iPS cell-derived cardiomyocytes

READ APPLICATION NOTE ►

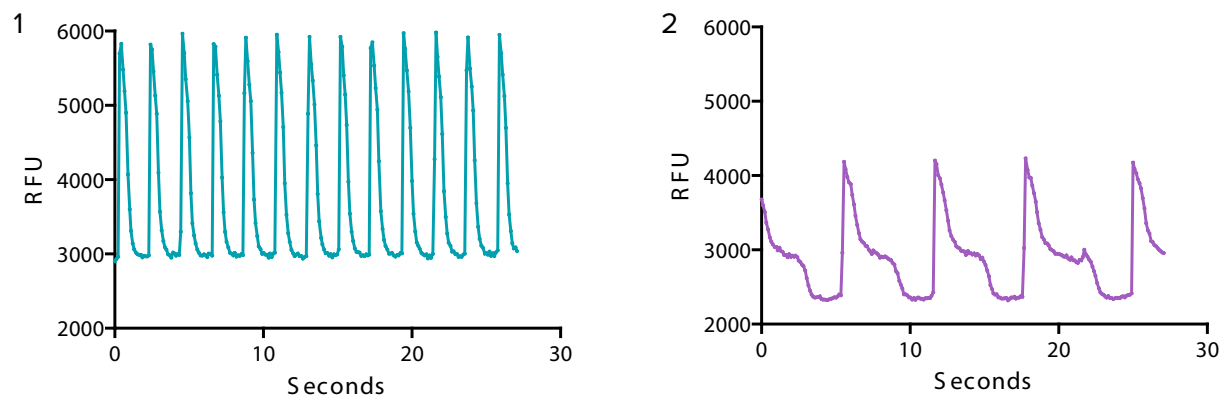


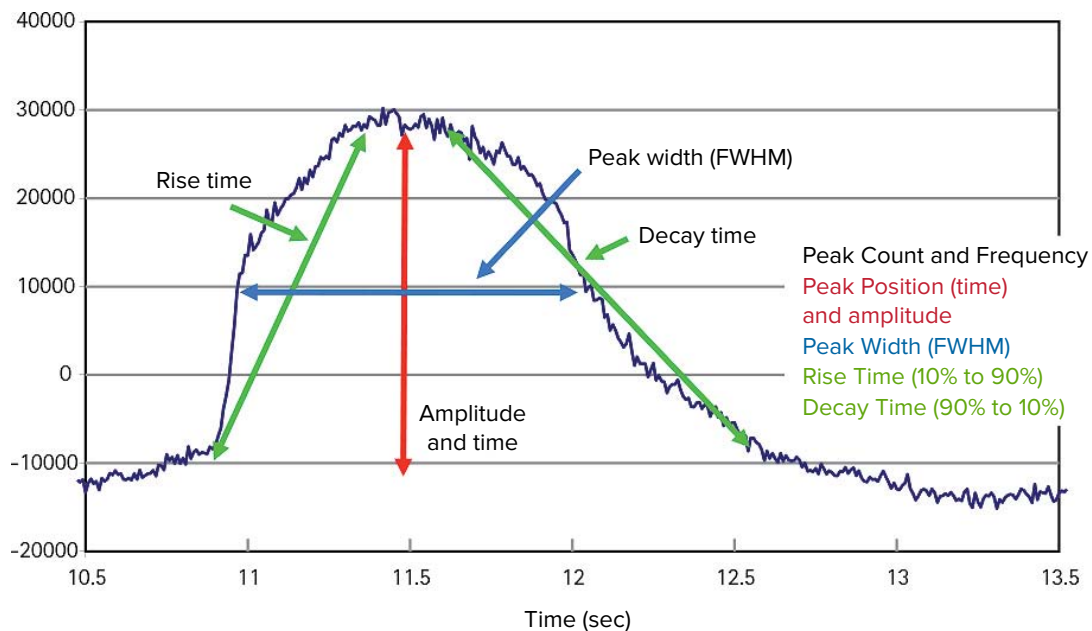
Figure 1. Calcium signal oscillation. In the experimental control, calcium signal oscillation reflects changes in cytoplasmic calcium concentration in Cor.4U iPS cell-derived cardiomyocytes measured with the EarlyTox™ Cardiotoxicity Kit on the FLIPR system. **Figure 2. Cisapride is a hERG blocker and causes a distinctly different beat pattern compared to the control in Figure 1.**



High throughput cardiotoxicity assays using stem cell-derived cardiomyocytes

READ APPLICATION NOTE ►

- Earlier prediction of compound toxicity and efficacy
- Robust, high throughput, biologically relevant assays
- Fast, simplified data analysis for compound prioritization



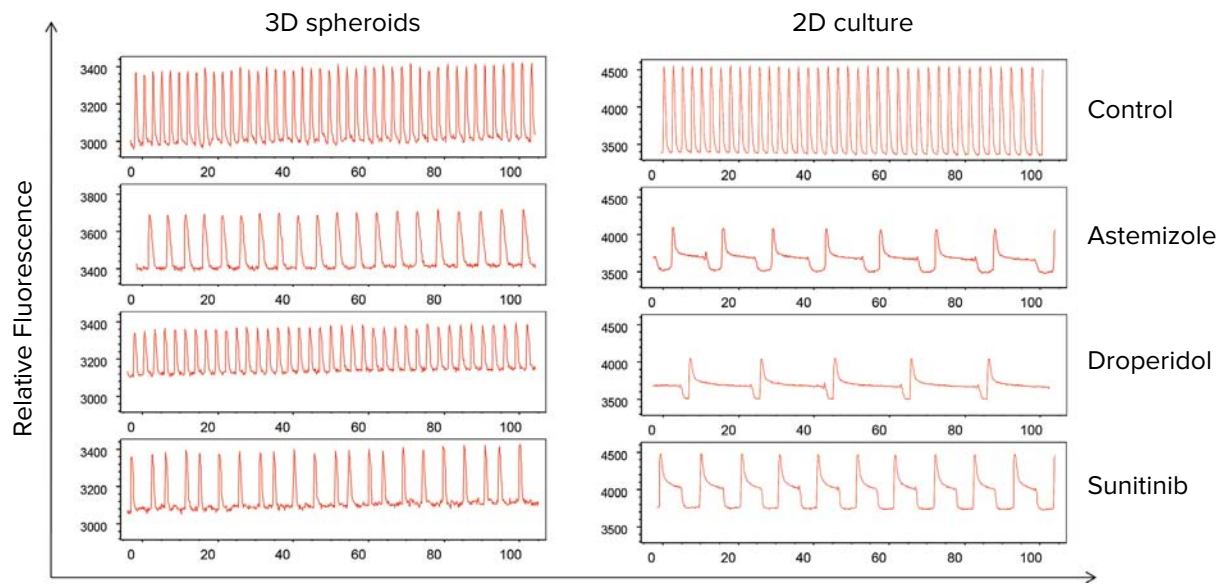
Software analysis output parameters. Output parameters available to the user in ScreenWorks® Peak Pro™ Software.



In vitro assessment of drug effects on human iPSC-derived cardiac spheroid cultures

[READ POSTER \(Direct PDF download\) ►](#)

- 3D cardiac spheroid models were generated using human iPSC-derived cardiomyocytes
- Responsiveness of the 3D culture Ca^{2+} flux assay was demonstrated to a panel of known cardioactive and cardiotoxic chemicals.
- A comparison of 3D versus 2D assay formats indicated altered pattern for Ca^{2+} flux waveforms, in particular for potassium channel blockers.



Comparison of the Ca^{2+} flux measurements between 3D spheroid and 2D cultures of cardiomyocytes treated with select compounds. Dose response curves for select compounds after 24 hours exposure are based on changes in peak count. EC_{50} values were shifted toward greater concentrations in 3D.

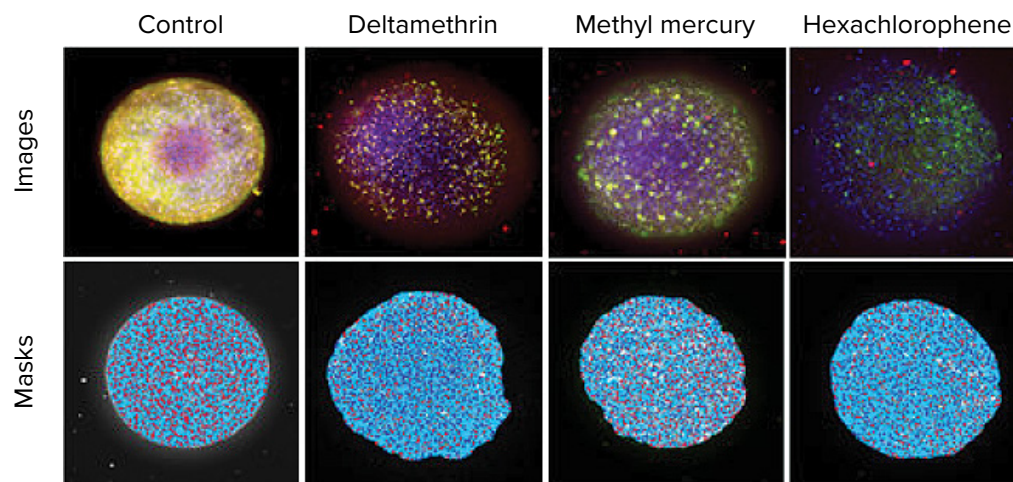


Functional and mechanistic neurotoxicity profiling using human iPSC-derived neural 3D cultures

[READ POSTER ►](#)

[READ PAPER ABSTRACT \(Full text available for a fee at Oxford Academic\) ►](#)

- We developed the methods and demonstrated feasibility of the iPSC-derived StemoniX[®] microBrain[®] 3D Assay Ready neural cultures for evaluation of compound effects
- Expected functional responses were demonstrated using known neuromodulators
- The assay can be used for testing compound effects and screening for neurotoxic chemicals



Nuclei—Hoeschst nuclear stain, shown in blue. Viability—Calcein AM, shown in green.
Mitochondria—MitoTracker Orange, shown in orange.

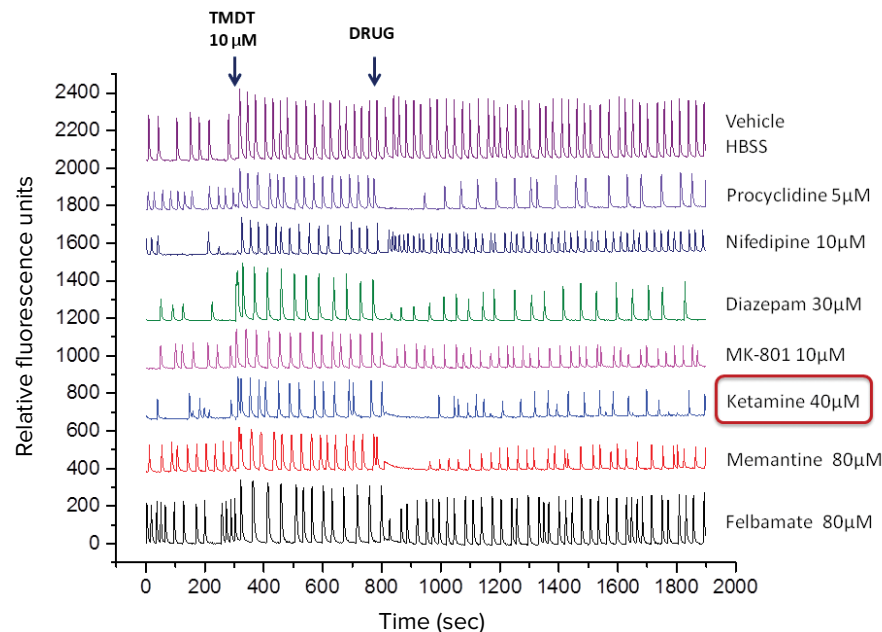
Composite projection images of neural spheroids. Spheroids were treated with 30 μM of indicated compounds for 24h, then stained with a nuclear stain (Hoechst 33342), viability stain (Calcein AM), and mitochondria potential dye MitoTracker Orange CMTMRos for 2 hours (2 μM , 1 μM , and 0.5 μM respectively). Spheroids were imaged with the DAPI, FITC, and TRITC, 10X Plan Fluor objective, imaged using z-stack of confocal images (30 images, 15 μm apart). Maximum projection images were analyzed using custom module editor for detection of spheroid size and shape, and also count of positive and negative cells in spheroid. The image show nuclei (blue), Calcein AM stain (green), and mitochondria (orange). The mask showing spheroids in blue, nuclei of Calcein AM positive cells in red, negative cells in blue.



Calcium flux assay for *in vitro* neurotoxicity studies and drug screening

READ APPLICATION NOTE ►

- Monitor intracellular calcium oscillations in primary neuronal cultures in real time
- Determine agents' potencies using Peak Pro analysis algorithms
- Compare agents based on their unique effects on calcium oscillation pattern in cultures
- Analyze peak frequency, amplitude, total area under the curve, and more



Representative kinetic traces using simultaneous injection of various drugs to the cells.

Baseline calcium oscillation read (0 to 300 seconds), tracings after addition of TMDT at 300 seconds followed by addition of various drugs at 800 seconds) are indicated. Note that TMDT (10 μ M) markedly increased the peak amplitude and thus the total intracellular calcium levels, as expected. Addition of pharmacological agents, applied at different concentrations displayed varying efficacies in reversing TMDT-induced effects upon oscillation pattern. OriginPro 2016 Software (OriginLab Corporation, MA) was used to draw all the plots in one graph.



Contact Us

Phone: +1.800.635.5577

Web: www.moleculardevices.com

Email: info@moldev.com

Check our website for a current listing of worldwide distributors.

Regional Offices

USA and Canada +1.800.635.5577

United Kingdom +44.118.944.8000

Europe* 00800.665.32860

China (Beijing) +86.10.6410.8669

China (Shanghai) +86.21.3372.1088

Hong Kong +852.3971.3530

Japan +81.3.6362.9109

South Korea +82.2.3471.9531

**Austria, Belgium, Denmark, Finland, France, Germany, Ireland, Netherlands, Spain, Sweden and Switzerland*