FDSS/μCELL

Cardiomyocyte in-vitro Toxicity Assay

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iPS derived cell drug discovery in FDSS

Recently, studies of iPS cells (induced pluripotent stem cells) have made a huge impact in the drug discovery field. Currently, human iPS cell (hiPSC) derived various specific types of cells such as cardiomyocytes and neural cells are now widely available commercially, and the screening of chemical compounds for drug discovery using these hiPSC-derived is possible. Screening using hiPSC-derived cells is expected to provide more effective and easy way to evaluate the pharmacological and toxic effects of test compounds in cell-based assays.

HAMAMATSU has developed new functions for the FDSS/µCELL which allows the measurement and analysis of calcium transients in hiPSC-derived cardiomyocytes. This is useful for in vitro toxicity screening using human cardiomyocytes, particularly at the early stage of drug development.

FDSS®/µCELL

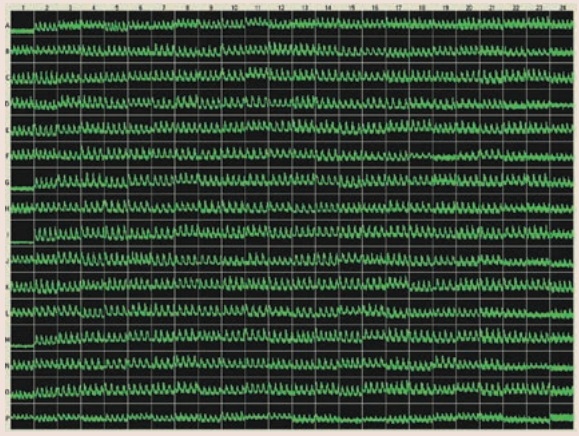
The FDSS/µCELL is a kinetic plate reader with an integrated dispensing head and imaging-based detector. Simultaneous dispensing into the entire 96/384 well plates and simultaneous detection of the kinetics of the fluorescence or luminescence intensity allow quick measurements with no time lag for the 96/384 well plate. The technologies employed in the FDSS series are integrated into a compact body, enabling simple-to-use operation, suitable for assay development or in researching basic cell-based kinetic assay.

Feature

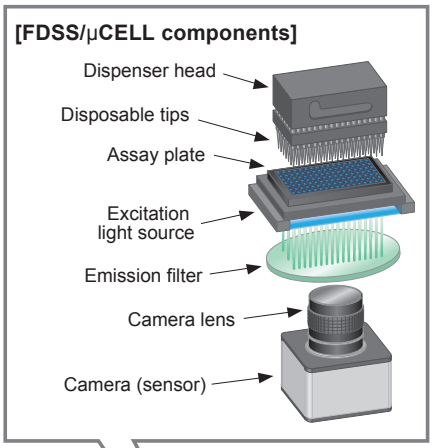
- **Small footprint, affordable, easy-to-use**
- **Simultaneous dispense and imaging whole 96/384 plate**
- **Dedicated optics to measure all well uniformly**
- **Long life and stable LED light source**
- **2 wavelength measurement options**

Ca^{2+}-transient measurements in human iPS-derived cardiomyocytes

Cell: iCell® Cardiomyocytes (Cellular Dynamics International)
Probe: Fluo-8/AM



FDSS/µCELL is capable of measuring Ca^{2+}-transients in iPS/ES-derived cardiomyocytes in 96/384-well plate format.



New Functions

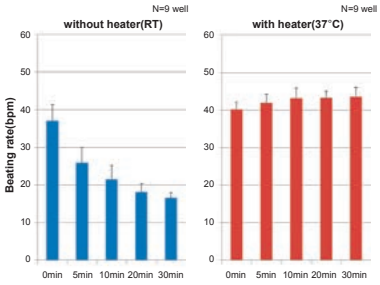
- **Temperature control with Heater Unit for stable beating of cardiomyocytes**
- **High speed data acquisition to accurately measure calcium oscillation (calcium transients) in cardiomyocytes.**
- **Software for analysis of calcium oscillation waveforms**

Above three options are developed to have more reliable results from the cardiomyocyte assay. Equipping with all of these options provide efficiency to compound toxicity study in early drug discovery stage.

New Option 1

Heater unit A11529-15

The Heater Unit is designed to maintain a stable temperature of all wells in a microplate at +35 °C to +37 °C. The beating of iPSC-derived cardiomyocytes is very sensitive to temperature and easily looses stability at room temperature. The heater unit greatly improves the stability of beating.



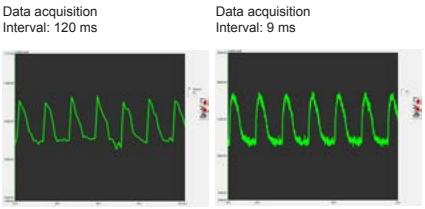
Cell: iCell® Cardiomyocytes (Cellular Dynamics International)

The above graph shows the changes in the beating rate of human cardiomyocytes in a microplate on the FDSS/µCELL during 30 minute incubation. Without the heater unit (left column, at room temperature), the beating rate gradually decreased with time and the rate dropped by half after 30 minute incubation. In contrast, when the well temperature was maintained at +37°C using the heater unit (right), the beating rate was unchanged even after 30 minute incubation.

New Option 2

FDSS Software option High Speed Acquisition option U8524-11

The High Speed Data Acquisition option for the FDSS/µCELL can acquire images with very short interval times (approx. 10 ms). To accurately measure the calcium oscillation in cardiomyocytes requires such high speed.



The above graph shows the fluorescent intensity change (calcium concentration change) in cardiomyocytes in a well, which were measured with 120 ms (left) and 9 ms (right) sampling intervals respectively. The main difference between the measurements with the two sampling rates is the time from the resting calcium concentration level (bottom) to reaching to the maximum calcium concentration (peak). It is shorter when measured with 9 ms intervals, which shows you may miss the accurate peak point in measurements with 120 ms sampling intervals. Shorter sampling intervals enables us to measure calcium oscillation more accurately.

New Option 3

FDSS Software option Waveform Analysis software for cardiomyocyte U8524-12

After measuring the calcium oscillation in cardiomyocytes with the FDSS/µCELL, you need to analyze the data. The new FDSS analysis software allows quick and easy analysis of the waveform of calcium oscillation.

Above is the capture of the beat analyzing software. This software is launched from FDSS software. Open the data with FDSS software and show the range to analyze. Then press the button to launch this software. 16 parameters can be analyzed by this software.

Analysis Software for waveform of calcium oscillation in cardiomyocytes

Support your analysis with multiple parameters (e.g. peak number) of calcium oscillation in iPS/ES-derived cardiomyocytes.

Feature

- **Visualize and analyze the calcium oscillation in cardiomyocytes.**
- **Auto-setting and visualized setting configuration.**
- **Flexible settings for various type of waveform.**
- **16 analysis parameters available.**

Parameters

(1)	**Peak number (Total, BPM)**
(2)	**P-P time [ms] (Ave, Std, Max, Min)**
(3)	**Ratio (Ave, Std)** *Ratio = (AMP + RMP) / RMP
(4)	**AMP (Ave, Std)**
(5)	**RMP (Ave, Std)**
(6)	**Slope (Ave, Std)** Rising Slope: Slope from bottom to peak Falling Slope: Slope from peak to bottom peak bottom setting can be selected 0 % - 100 %, 10 % - 90 %, 20 % - 80 %, 30 % - 70 %
(7)	**Area under curve (Ave, Std)**
(8) to (16)	**PWD (PWD10 to 90)[ms] (Ave, Std)**

Fig1

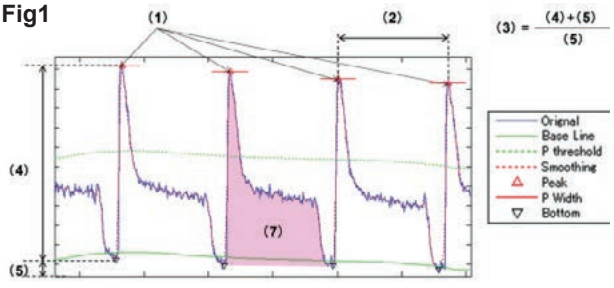
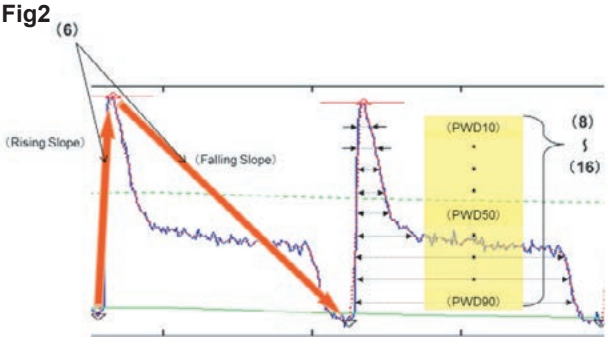
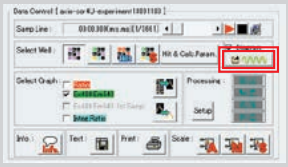


Fig2

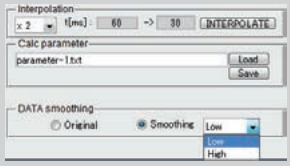


Procedure

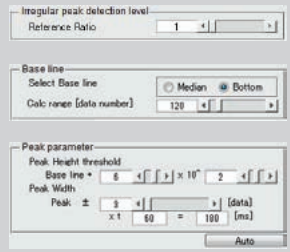
1 FDSS data loading
Press the button in the FDSS software to launch the analysis software and the data shown in current range is transferred to be analyzed.



2 Data preparation
●Interpolation
Duplicate the sampling number to interpolate the missing sampling for low sampling rate data.
●Smoothing
Smoothing is to remove the noise in the waveform data, useful for very high sampling rate data.

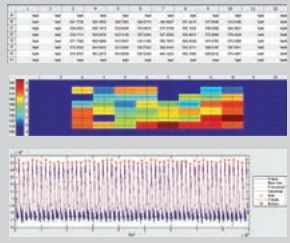


3 Analyze settings
●Irregular peak detection level
Threshold to judge irregular beating
●Base line
Select median or bottom value to create base line, and its range.
●Peak parameter
Configure the threshold level from the baseline to detect peak, and to set the width range for one peak.
●AUTO
Automatic settings are available.

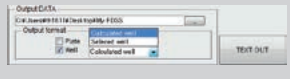


4 Analanalyze
Select the necessary parameter and analyze for the whole plate or well by well

5 Results
Displays data in plate format, waveform for the selected well
●Analyzed data display in plate format
●Colored plate to visualize the data value high/low among the wells.
●Analyzed waveform display for the selected well



6 Text out results
Exporting data in text file with plate format, and the data of each well.



Experimental Protocol

Standard protocol for calcium ion assay using iPS-derived cardiomyocytes are determined by the cell manufacturer. Please consult your cell manufacturer for details.

1 Plating cells in 96/384-well microplates

Step1 **Coat the plate with the material described in the cell provider's instruction manual.**

Step2 **Thaw, plate and culture the cells according to the cell provider's instruction manual.**

Please refer to below site, for the latest calcium transient protocol.
https://www.reprocell.com/en/products/cell-biorepository/cardiomyocyte/reprocardio2

2 Ca^{2+} dye loading to cells

Step1 **Prepare the Loading buffer at +37°C**

Loading Buffers

HEPES-Hank's Balanced Salt Solution (calcium, magnesium) (pH7.4)	Thermo Fisher Scientific #14025-092 Thermo Fisher Scientific #15630-056
2 µM Fluo8-AM	AAT Bioquest #21083
0.05 % Pluronic F-127	Thermo Fisher Scientific # P-6866
1.25 mM Probenecid	Sigma #P8761

Step2 **Loading**
· Remove the culture medium
· Add 80 µL/well of +37°C Loading buffer prepared in Step 1
· Incubate the cells for 1 hour at +37°C in 5 % CO_2

Step3 **Wash out**
· Remove Loading buffer
· Add 100 µL/well of +37°C HEPES-Hank's Balanced Salt Solution

NOTE: This dye loading protocol is just one example you may need to optimize it to have better performance in your experiments.

3 FDSS Data Acquisition / Data Analysis

Step1 **Instrument Set up**
· Turn on the system 30 minutes before the experiment to cool the camera and to warm the stage up to +37°C
· Launch FDSS software

Step2 **Protocol Setting**
· Set the interval to 10 ms to 30 ms, and configure the sampling number
· Set the dispense parameter if dispense is necessary in the protocol

Step3 **Data acquisition and analysis**
· Start assay with the configured setting protocol
· Open the measured data and analyze them

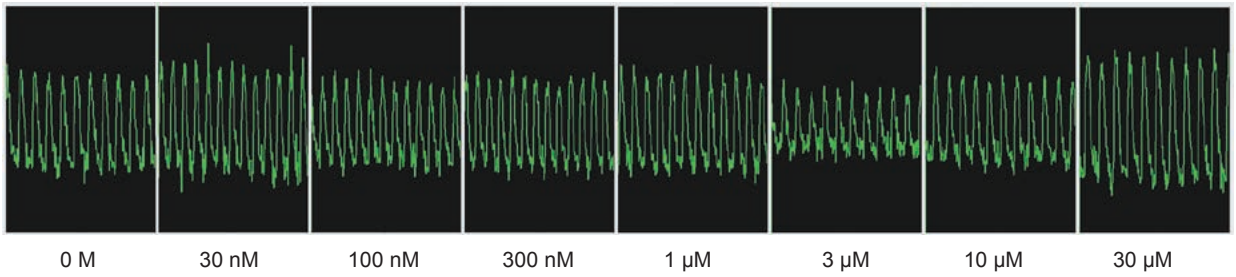
Measurement and Analysis examples

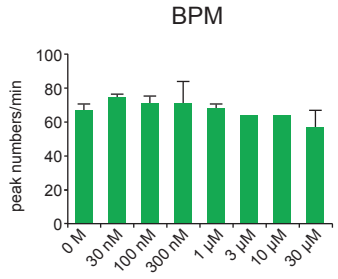
In-vitro toxicity study examples using iPS/ES derived cardiomyocytes

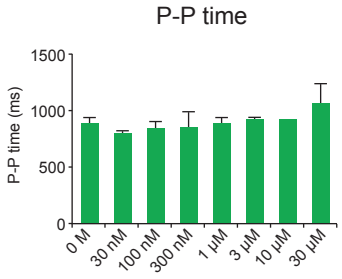
Evaluated Compound List

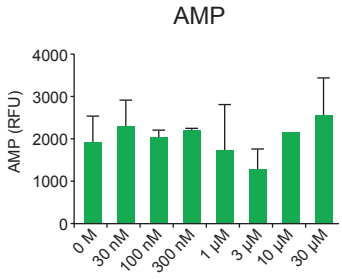
Compound	Description	Arrythmia	Contractability	Concentration of Compound
Aspirin	Known compound as inhibitor of cyclooxygenase which does not show cardiotoxicity in this experiment			30 nM to 30 μM
E-4031	Known compound for hERG potassium channel blocker	●		10 nM to 10 μM
Isoproterenol	Known compound which sitimulates cardiomyocyte influencing β-receptor		●	30 nM to 30 μM

Compound 1 Aspirin

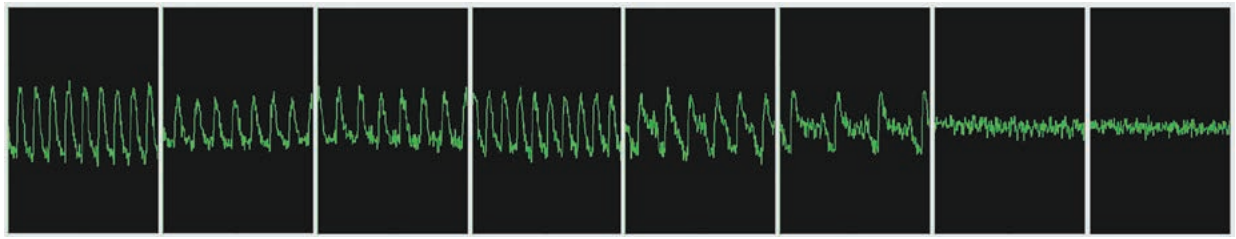


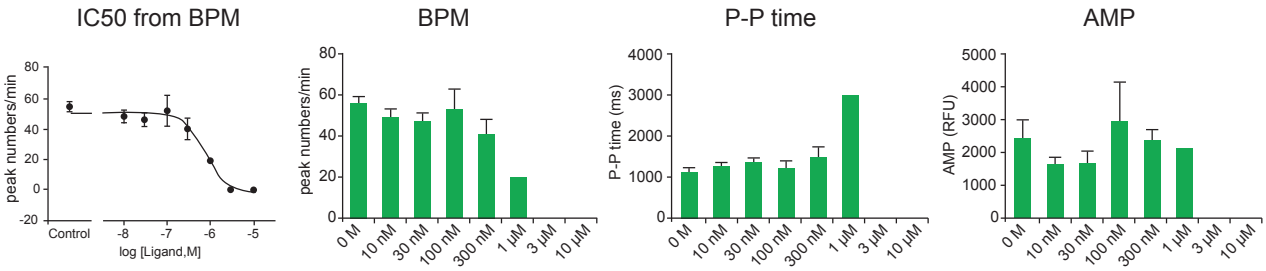




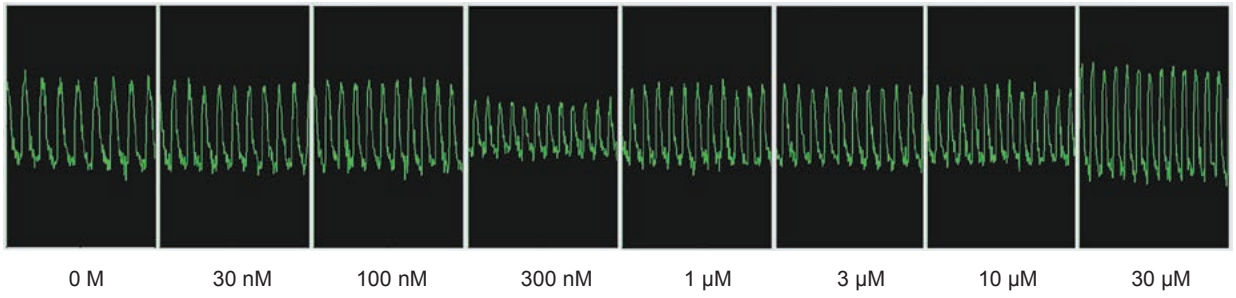


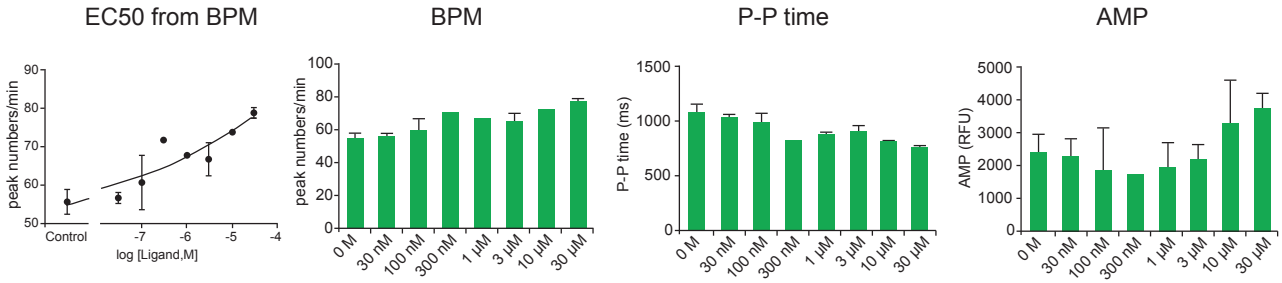
Compound 2 E-4031





Compound 3 Isoproterenol





Basic System Configuration for measurement

For Kinetic measurement, either 96/384 dispenser head or EFS pacing system is required

Base unit	C7903-11, U8524-01A, U8524-03A, A11529-01A, A11529-02, M11031-02 A11529-04, A11529-05, A11529-15, U8524-11	Standard configuration for Cardiomyocyte package
Sensor	C9100-23B M7791-19 A6402	EM-CCD camera with Frame grabber board and cables, C mount lens
Light Source array unit (B, G)	L11601-06	Light Source for Fluo-4 and FMP, ex1: 470 nm/ ex2: 530 nm, em1: 540 nm/ em2: 593 nm
FDSS Software option Waveform Analysis Software for Cardiomyocyte	U8524-12	BPM, P-P, Amplitude, Slope, Area Under Curve, PWD (10, 20, 30, 40, 50, 60, 70, 80, 90)

OPTIONS

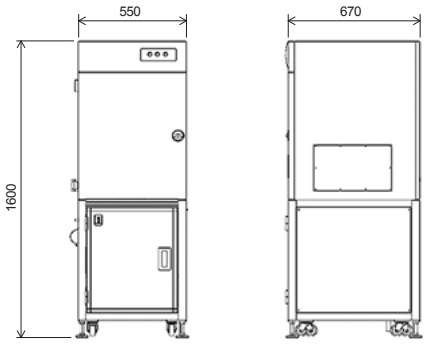
EFS pacing system	M13040-01	Stimulation Voltage: 0 V to 20 V, Frequency: 0.1 Hz to 50 Hz, Pulse Width: 1 ms to 100 ms, Number: 1 to 1000 times Caution Notice: The FDSS/µCELL EFS system should not be used for optically detecting / monitoring change in transmembrane potential of the cells. The FDSS/µCELL EFS system should not be used on any cell or cells in which the user or anyone else has expressed target ion channels
Dispensing unit (96 tip type)	A10118-24	96 ch Dispenser Head, for kinetic measurement
Dispensing unit (384 tip type)	A10118-26	384 ch Dispenser Head, for kinetic measurement
Washing unit	A11529-09	Wash vat, in/out pump, tubes, wash/waste tanks
Chimney plate (96 tip type)	A11529-12	Chimney Plate for 96 dispenser head wash
Chimney plate (384 tip type)	A11529-13	Chimney Plate for 384 dispenser head wash

Consumables

96 black tip (10 racks) for FDSS7000/µCELL	A8687-32A	Disposable plastic tips for 96 well plate format assay, contains 10 racks
384 black tip (10 racks) for FDSS7000/µCELL	A8687-62A	Disposable plastic tips for 384 well plate format assay, contains 10 racks

Dimensions

Dimensions/Weight (Main unit)	550 mm (W) x 1600 mm (H) x 670 mm (D) / approx. 200 kg
Dimensions/Weight (Data Analysis unit)	300 mm (W) x 500 mm (H) x 500 mm (D) / approx. 20 kg *When using our standard computer rack which is only available in Japan Only. Please refer to the local Hamamatsu representative for the computer rack prepared locally.



[Dimensions (Unit: mm)]

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