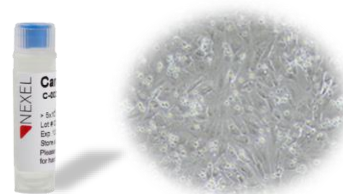
Application note

NEXEL

Measurement of calcium transient in monolayer cultures of Cardiosight®-S



Introduction

The Cardiosight®-S, a highly pure iPSC cardiomyocytes manufactured by NEXEL through proprietary induced differentiation technology, is used in the comprehensive in vitro proarrhythmia assay (CiPA) for its ability to detect arrhythmia caused by test compounds using electrophysiological test equipment and has been proven to be a suitable cardiomyocyte for the evaluation of cardiac safety.

One of the main topics of cardiovascular research is the study of calcium handling, since even small changes in intracellular calcium concentration can alter the functional parts of the cell.

These calcium ions (Ca^{2+}) are key second messengers in Excitation-Contraction(EC) coupling of cardiomyocytes, which play a vital role in cellular functions. Therefore, calcium measurement enable *in vitro* screening of compound efficacy and toxicity in human cardiac myocytes.

This application note describes how to quickly and easily use the Cardiosight®-S for 2D calcium transient measurements with a micro plate reader. In addition, representative drugs known to affect cardiomyocytes were applied to the Cardiosight®-S to determine how calcium flux varies with each drug.

Workflow

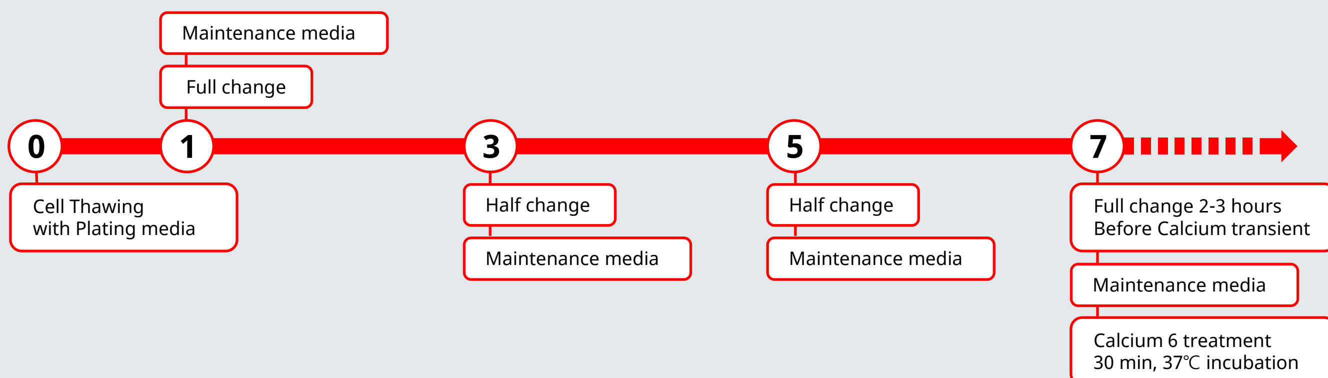


Table 1. Timeline of the Workflow from Day 0.

Note: An alternative workflow may be acceptable. Contact NEXEL's Technical Support for more information.

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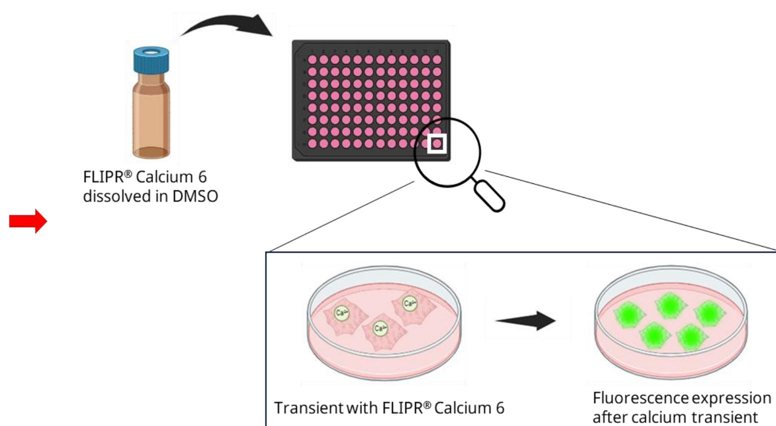
Cardiosight®-S (#C-002) and CMS-A media kit

hiPSC culture Thaw & maintenance

- Cells seeding with Matrigel coating
- 50,000 cells/well
- Full media change on Day 1 to maintenance media and half media exchange every 48 hrs



Required Consumables

Item	Vendor	Catalog number
Cardiosight® -S Cardiomyocytes	NEXEL	C-001 C-002
Cardiosight® -S Advanced Media	NEXEL	CMS-001A CMS-002A
FLIPR® Calcium 6 Assay Kit (Component A)	Molecular Devices	R8195
Dimethyl Sulfoxide (DMSO)	Sigma Aldrich	D2650
Corning® CellBIND® 96-well Flat Clear Bottom Black Polystyrene Microplates, with Lid, Sterile	Corning	3340

Method

Cell culture

The cardiomyocytes used in this study were manufactured and provided by NEXEL Co., Ltd. Cells were thawed and cultured according to the Cardiosight®-S User Guide. 50,000 cells were plated in 96-well black plate and cultured for 7 days.

For media changes, prepare the required amount of maintenance media and place it in the R.T. at least 30 minutes before the media change to allow it to warm up. On Day 1, replace all of the media with fresh, and then culture the cells to Day 7 by replacing half the media every other day.

Calcium dye preparation

Reconstitute component A of the FLIPR® Calcium 6 kit (Molecular Devices, Sunnyvale, CA) according to the manufacturer's instructions. Dissolve the Calcium 6 dye in Dimethyl sulfoxide (DMSO) to make it 200X and store at -20℃, dissolve and use as needed. It is recommended to refer to the 'Workflow'.

Drug preparation

A total of two drugs, Isoproterenol and Nifedipine, as well as DMSO as a negative control, were prepared for the calcium transient assay (Table 2).

Each drug was treated as a single treatment and was diluted in the culture medium at a ratio of 1:100 for a final concentration of 1000X, and then added to the cells so that they were 1/10 diluted upon drug treatment.

Calcium transient assay

On the day of the assay, replace with 200 μl of maintenance medium per well prior to 2-3 hours before the assay.

Prepare the Calcium 6 dye by mixing it with the culture medium in a 1:20 ratio. Then replace 20 μl of culture medium in each well with 20 μl of Calcium 6 and incubate at 37℃ for 30 minutes. After 30 minutes of incubation, measure intracellular calcium flux using a SpectraMax iD3 set to 37℃ (Baseline).

After the baseline measurements, the cells were treated with drugs known to affect cardiomyocytes and incubated at 37℃ for either 15 or 30 minutes. Calcium transient is measured in the same way as baseline.

Results

Calcium transient analysis

Treatment of drugs may affect the degree of influx and efflux of intracellular calcium ions. To investigate the effects of drug treatment, calcium transient assay was performed using a set of representative agonist and antagonist drug, Isoproterenol and Nifedipine, which are known to affect the heart.

Dimethyl Sulfoxide was used as the solvent for both drugs, and 0.1% DMSO was used as a negative control (NC). For the treatment of Nifedipine, a single concentration was administered and left to incubate for 30 minutes at 37℃. After the incubation period, calcium transient was measured. Likewise, for the treatment

	DMSO	Isoproterenol	Nifedipine
Effect	Negative control	Positive inotropy effect	Ca^{2+} channel blocker
Concentration	0.1%	3 μM	0.1 μM

Table 2. Test drug concentration for 2D Calcium transient

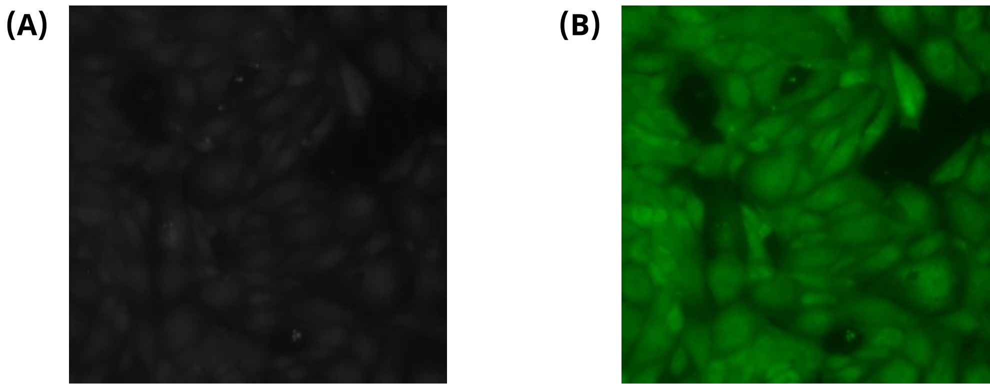


Figure 1. 2D calcium transient Image using fluorescence microscope.
Representative image showing intracellular calcium influx. The scale bar represents 200 μm. Calcium influx was measured by treating cells with Calcium 6 dye (green). **(A)** Image of the absence of intracellular calcium influx. **(B)** Images of active intracellular calcium influx.

of Isoproterenol, a single concentration was administered and left to incubate for 15 minutes at 37℃.

Identical to Nifedipine, calcium transient was recorded after the incubation period. The difference in the incubation period between Nifedipine and Isoproterenol is due to the fast-acting nature of Isoproterenol, which prompted for a relatively shorter period of incubation of 15 minutes.

Effect of all treated drugs, including the negative control, were evaluated across four parameters: Amplitude, Beat rate, Time to peak (Rising Time), and Peak to baseline (Falling time).

Negative control (NC), treated with 0.1% DMSO, exhibited a decrease of 10% or less in all four parameters, which were not significant.

For Isoproterenol, significant increase in the amplitude was observed. As for beat rate, time

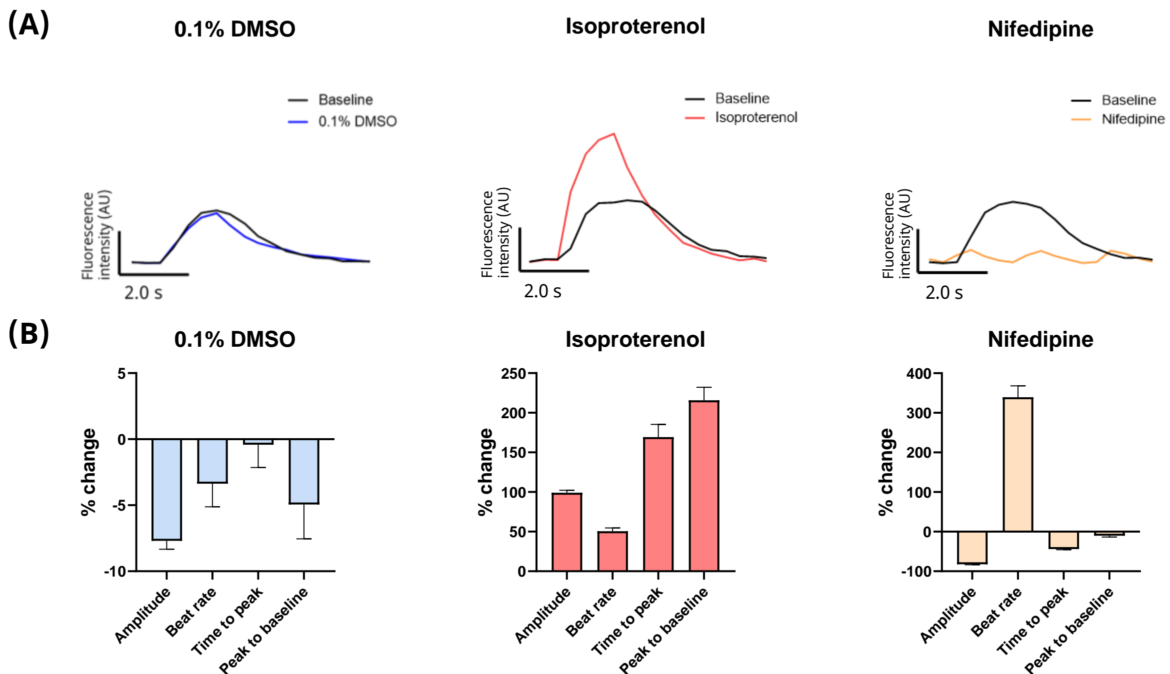


Figure 2. Representative traces and Reactivity for each drugs of Cardiosight®-S.
(A) shows a representative trace of the negative control (NC), 0.1% Dimethyl sulfoxide (DMSO), and the two drugs, Isoproterenol and Nifedipine, in that order. The negative control, 0.1% DMSO, and two drugs (Isoproterenol, Nifedipine) recorded 1) baseline before drug treatment, and 2) drug reactivity 15, 30 minutes after drug treatment. (B) Drug reactivity of Cardiosight®-S for each drug is shown in %change. Nifedipine and negative control (DMSO) were measured after cells were drug-treated for 30 minutes. Isoproterenol was measured after 15 minutes of drug treatment. There are four parameters: Amplitude, Beat rate, Time to peak, and Peak to baseline. For the negative control group treated with 0.1% DMSO, we found that all parameters were stable at around 10% or less. Isoproterenol showed high reactivity in Time to peak, Peak to baseline. It also showed high reactivity in Amplitude, approaching 100%, which was consistent with representative tracing results. Nifedipine showed a high reactivity of over 300% in Beat Rate, which was also consistent with representative tracing results.

to peak, and peak to baseline, it has shown a greater degree of change when compared to the baseline values.

Nifedipine, a calcium channel block known to affect extracellular field potential and contractile force, resulted in a significant increase in beat rate, recorded at over 300%. As for other remaining parameters, amplitude, time to peak, and peak to baseline all showed a tendency to decrease.

Conclusion

Calcium ions play key roles in regulating multiple biological processes in cells, ranging from but not limited to muscle contraction to the release of neurotransmitters. Therefore, the need for reliable fluorescent calcium-labeled dye is critical for studying many aspects of cell biology and screening compounds using phenotypic high-throughput assays.

Along with the need for the reliable fluorescent calcium-labeled dye, a reliable yet sensitive cell is required for the screening assay to be in full effect. Cardiosight®-S allow for a quick and easy determination of drug response to various compounds that affect cardiomyocytes, as evidenced by the results of intracellular calcium concentration visualized through the use of dye and microplate reader. With the right tool and cell, it can be expected that the combined platform of calcium transient assay dye and Cardiosight®-S can serve as a mean to screen and predict cardiac pharmacotoxicity *in vitro*.

Reference

1. Gilbert G, Demydenko K, Dries E, Puertas RD, Jin X, Sipido K, Roderick HL. Calcium Signaling in Cardiomyocyte Function. Cold Spring Harb Perspect Biol. 2020 Mar 2;12(3):a035428.doi:10.1101/cshperspect.a035428. PMID: 31308143; PMCID: PMC7050587.

2. Dewenter M, von der Lieth A, Katus HA, Backs J. Calcium Signaling and Transcriptional Regulation in Cardiomyocytes. Circ Res. 2017 Sep29;121(8):1000-1020.doi:10.1161/CIRCRESAHA.117.310355. PMID: 28963192.

3. Daily NJ, Santos R, Vecchi J, Kemanli P, Wakatsuki T. Calcium Transient Assays for Compound Screening with Human iPSC-derived Cardiomyocytes: Evaluating New Tools. J Evol Stem Cell Res. 2017;1(2):1-11. doi: 10.14302/issn.2574-4372.jesr-16-1395. Epub 2017 Jan 24. PMID: 28966998; PMCID: PMC5621642.

For questions about culturing our **Cardiosight®-S**, please contact NEXEL's Technical Support at

▪ Email: technical_support@nexel.co.kr
▪ Phone: +82-2-2088-8886

Caution: Since all experimental steps described in this application note are optimized for Cardiosight®-S, results cannot be guaranteed when carried out with different cells. NEXEL recommends the use of media and reagents listed in the application note, otherwise results may not be replicable and further technical support may be difficult.