

Application Note

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Cardiotoxicity of iCell[®] Cardiomyocytes Using the CyBi[®]-SELMA 96, 250 μ l Semi-automated Pipettor

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Key words: Automated liquid handling, iCell[®] Cardiomyocytes, cell-based assays

Introduction

The CyBi[®]-SELMA (CyBio AG, Jena, Germany) is a compact, user-friendly, semi-automated 96- or 384-tip pipettor that is well suited for low-throughput applications. Since the CyBi[®]-SELMA fits easily into a tissue culture hood, one attractive use is for media changes and compound dosing in cell-based assays.

iCell[®] Cardiomyocytes (Cellular Dynamics International, Madison, WI) are a human induced pluripotent stem (iPS) cell-derived cardiomyocyte cell line representing a pan population of atrial, nodal, and ventricular cells. These human cardiac cells are suitable for a wide variety of applications, including evaluation of the cardiac cytotoxicity of pharmacologically active compounds. In such assays, pipetting steps including serial dilutions, compound dosing, and cell feeding can be tedious, time consuming, and subject to inter operator differences in technique, accuracy, and reproducibility.

In this study, the CyBi[®]-SELMA was implemented for determining the cardiac cytotoxicity of a series of compounds on the viability of iCell[®] Cardiomyocytes, using the CellTiter-Glo[®] Luminescent Cell Viability Assay (Promega, Madison, WI). The user-friendliness, speed, and consistency of the CyBi[®]-SELMA in this assay vs manual pipetting was evaluated.



Figure 1: The CyBi[®]-SELMA 96, 250 μ l with deep well tips and 2 working positions in a sterile hood

Materials and Methods

The cardiotoxicity assay was performed on iCell® Cardiomyocytes as previously described¹. Cardiomyocytes were thawed according to manufacturer's instructions and were cultured on a gelatin-coated 96-well plate in iCell® Cardiomyocytes Maintenance Medium for 7 days. The CyBi®-SELMA 96, 250 µl with deep well tip tray was used for media changes every other day. Cells were switched to a serum-free medium for 24 hours before performing the assay.

Using the CyBi®-SELMA 96, 250 µl, serial dilutions of compounds with known toxicity (staurosporine, imatinib, sunitinib and rotenone) were applied to the iCell® Cardiomyocytes for 24 hours. Viability was measured using the CellTiter-Glo® Luminescent Cell Viability Assay. Method parameter settings for the CyBi®-SELMA 96, 250 µl are shown in Table 1.

Serial dilutions were created by manually pipetting 150 µl of compound (staurosporine and imatinib, 100µM; sunitinib, 300µM; rotenone, 300µM) into the first column of the microplate. Media (100 µl) was transferred into the other 11 columns using the CyBi®-SELMA 96, 250 µl. Ten 3-fold serial dilutions transferring 50 µl, with mixing, were performed across the columns of the microplate using the CyBi®-SELMA 96, 250 µl.

Devices

- » CyBi®-SELMA 96, 250 µl (CyBio AG, #OL 7001-26-200)

Reagents

- » iCell® Cardiomyocytes with Plating and Maintenance Media (Cellular Dynamics #CMC-100-110-001)
- » staurosporine (Sigma #S6942)
- » imatinib (Sigma #Z7042)
- » sunitinib (Sigma #PZ0012)
- » rotenone (Sigma #R8875)
- » CellTiter-Glo® Luminescent Cell Viability Assay (Promega #G7571)

Consumables

- » CyBi®-TipTray 96 -250 µl deep well sterile (CyBio AG, #OL 3800-25-659-S)
- » 96 Well Flat Clear Bottom Black Polystyrene TC-Treated Microplates (Corning #3603)

Table 1: CyBi®-SELMA 96, 250 µl parameter settings for Cell Feeding, Media Addition, and Dosing

Experimental Process	Cell Feeding	Media Addition	Dosing
CyBi®-SELMA Program	Pipetting	Reverse Pipetting	Serial Dilution
Aspirate vol	100 µl	110 µl	50 µl
Dispense vol	100 µl	100 µl	50 µl
Blowout vol	70 µl	70 µl	70 µl
Piston speed	25 µl/s	10 µl/s	40 µl/s
Options	Mix off	1 step	Mix 3x 50 µl 10 steps

Results and Discussion

All compounds assayed significantly reduced cell viability, with potencies characteristic for the four compounds as shown in Figure 2 and Table 2, respectively.

The CyBi®-SELMA performed the viability assay on the iCell® Cardiomyocytes accurately, with EC₅₀ values generated for the four compounds in line with previous observations¹. The CyBi®-SELMA system significantly reduced media exchange time and minimized the exposure of the test system to ambient temperatures. In addition, the CyBi®-SELMA was user-friendly and well-suited to perform media exchanges and compound dosing using iCell® Cardiomyocytes.

Table 2: EC₅₀ values for compounds tested

Compound	24 h EC ₅₀
staurosporine	2.7e-07 M
imatinib	1.7e-05 M
sunitinib	4.3e-06 M
rotenone	8.0e-09 M

The wide range for pipetting speed adjustment (2 µl/s to 245 µl/s) allowed slowing down the aspiration and dispensing speed sufficiently so that the cell layer was not disturbed during automated pipetting and no bubbles were created with media dispensing. Height settings could be finely adjusted for optimized aspiration and dispensing to the cell monolayer.

Cell Titer-Glo® Cell Viability Assay

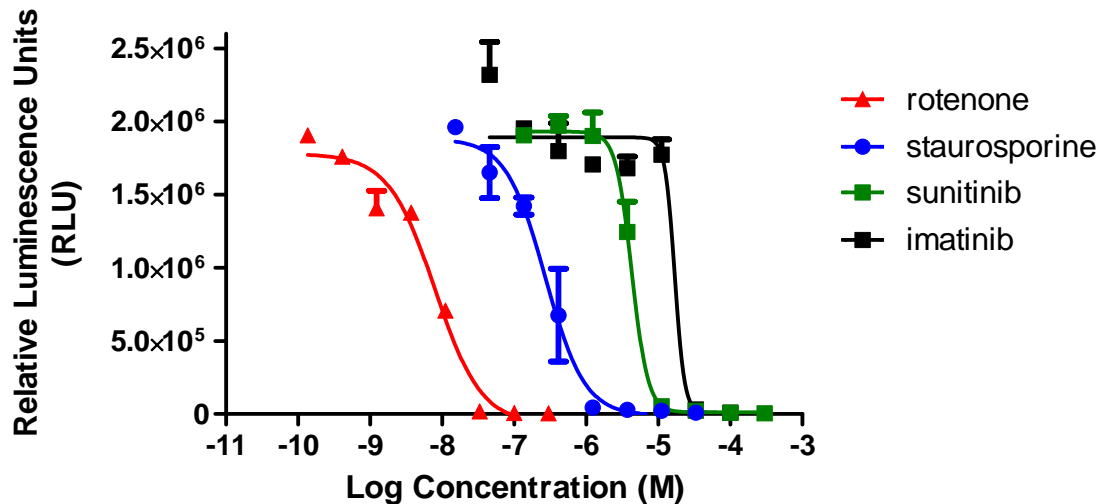


Figure 2: Cardiac cytotoxicity of different compounds in the Cell Titer-Glo® Cell Viability Assay, cell feeding, media addition and compound dilution were performed with the CyBi®-SELMA 96, 250 µl

The CyBi®-SELMA allows these method parameter settings to be saved for subsequent use, giving excellent consistency between runs and between operators. This was especially useful for performing serial dilutions in the cell culture plate, where differences in pipetting height between columns can disturb the cell layer or produce pipetting errors when done manually.

Overall, the CyBi®-SELMA was an effective and user-friendly tool for automating the viability assay on iCell® Cardiomyocytes, producing excellent results and pipetting consistency throughout the assay.

References

(1) iCell® Cardiomyocytes Application Note: Assaying Cell Viability, Cellular Dynamics International, Madison, WI.
<http://www.cellulardynamics.com/products/lit/index.html>

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