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Application Note

Automated Cell Dispensing into 1536-Well Microplates for HTS

Using the MicroFlo™ Select to Seed Tissue Culture Cells

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Today's HTS demands have moved screening assays towards high well density plates as well as placed more emphasis on cell-based assays in lieu of the conventional biochemical determinations. High density plates allow more samples to be assayed simultaneously, conserve reagents and lower assay costs. The use of 1536-well microplates for cell based assays requires the use of accurate and reliable automation in order to dispense uniform numbers of cells to each microplate well in a volume of a few microliters. Here we describe the use of the peristaltic pump-based MicroFlo™ Select to dispense cells into 1536-well microplates.

Introduction

High throughput screening has begun a ? shift from novel chemical entities to novel biological entities resulting in more cell-based assays while concurrently moving towards the use of high well density plates such as the 1536-well microplate. These plates require significantly less reagent than lower density plates (e.g 96- and 384-well), while allowing greater numbers of samples or experimental conditions on the same microplate. The small size and low volume requirements of the wells of a 1536-well plate make the accurate and repeatable dispensing of fluids a challenge. The dispensing of cells in suspension also requires that the solutions remain sterile and that the cells remain viable. Here we describe the use of the MicroFlo™ Select peristaltic pump dispenser to seed 1536-well microplates with tissue culture cells.

Materials and Methods

CellTiter-Glo® reagent was purchased from Promega Corp. (Madison WI). Solid white 1536-well microplates (catalog # 3727) and clear bottom, black sided 1536-well plates (catalog # 3893) were obtained from Corning (Corning, NY). Cell lines (h-mesothelioma or CHO-M1 stock cultures were trypsin-dispersed, counted using a hemocytometer and resuspended in fresh media at a concentration of 200,000 cells per mL (200 cells/µL). Different numbers of cells (based on dispense-volume) were dispensed into 1536-well microplates using a MicroFlo Select (BioTek Instruments, Winooski, VT). Media was added as needed such that all wells contained a total volume of 4 µL. Cell suspensions were allowed to attach at 37°C 5% CO_2 for 4-6 hours. After attachment 4 µL of CellTiter-Glo reagent, previously reconstituted, was added to all wells and the plate incubated at room temperature for 10 minutes (Figure 1).

The luminescence was then determined using a Synergy 2 Multi-Mode Microplate Reader (BioTek Instruments) as described previously [1].

For experiments involving luminescent quantitation using CellTiter-Glo reagent, cells were dispensed into solid white plates. Experiments involving photographs used clear bottom black-sided plates.

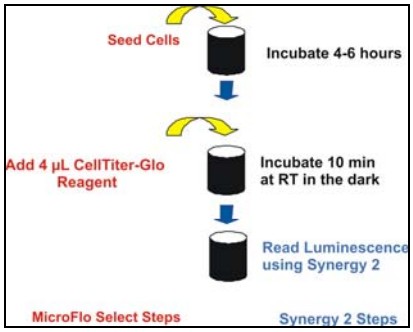

Figure 1. CellTiter-Glo Assay Procedure

Results

Equal numbers of cells (800 cells/well) were dispensed into all the wells of a 1536-well microplate and the uniformity assessed using a CellTiter-Glo assay. The CellTiter-Glo assay, which measures the presence of ATP, produces a luminescent signal that is proportional to the number of viable cells present.

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As demonstrated in Figure 2 the luminescent signal is consistent across the entire plate, which indicates that the MicroFlo is capable of delivering consistent volumes of both cell suspensions as well as CellTiter-Glo reagent.

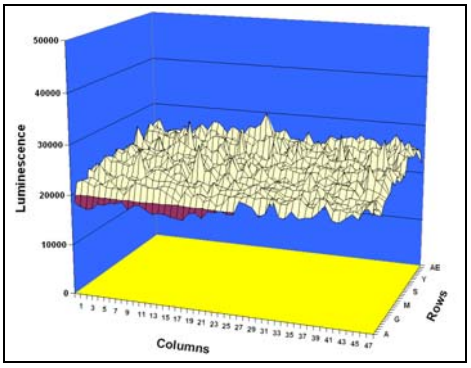


Figure 2. **Uniformity of Dispensing into 1536-well Microplates.** Surface plot of the data generated from a CellTiter-Glo luminescent assay. A MicroFlo Select was used to dispense 4 µL of CHO-M1 cell suspension followed by 4 µL of CellTiter-Glo reagent to all the wells of a 1536-well microplate.

The CellTiter-Glo assay is very sensitive, thus capable of detecting small numbers of cells. As seen in Table 1, the signal generated by 800 cells can be distinguished from the zero cells control wells with very high Z' values.

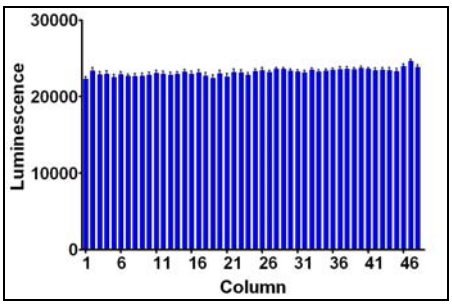
Cell Number	Mean	STD	Z' Factor
0	10	5	------
800	23210	1870	0.75

Table 1. Whole plate Dispense-statistics. The mean, standard deviation and Z' value for CellTiter-Glo data generated from two 1536-well plates. One plate received 4 µL of media only while the second received 4 µL of cell suspension containing 200 cells/µL.

When column and row constancy of a plate is examined, very little difference between either is observed (Figure 3). The MicroFlo, which has eight dispense tips, was programmed to dispense in a serpentine fashion down the long axis of the microplate. The mean values of columns on a plate represent an average of all eight tips. The pattern for rows is such that an entire row is dispensed with an individual tip. While the signal consistency on a row basis is not as tight as that observed with the column measurements, there is no discernable pattern indicating the variation is not related to any specific tip.

When cells are seeded into 1536-well plates they are uniformly dispersed within the wells and remain viable for at least 24 hours. As seen in Figure 4, H-mesothelioma cells are evenly dispersed, sterile and viable 24 hours after being dispensed with the MicroFlo Select.

A



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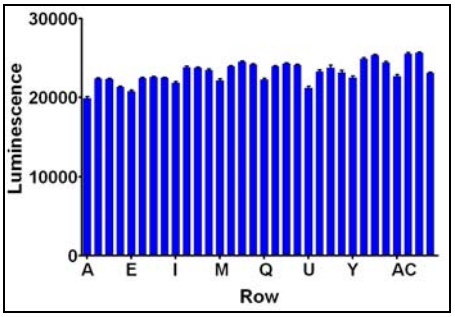

Figure 3. Row and Column Consistency. The Mean and SEM of each column (A) and row (B) of a 1536-well CellTiter-Glo assay plate. Note that each data point for column and row consistency represents the mean and SEM for 32 and 48 data points respectively.

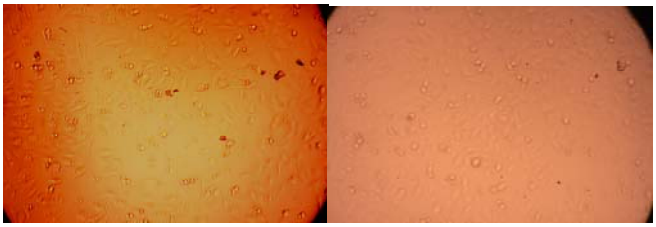

Figure 4. Representative images of H-mesothelioma Cells Dispensed into 1536-well plates using a MicroFlo Select. Digital light transmission images were taken with a Zeiss inverted microscope configured with a Nikon camera.

The MicroFlo Select can also be used to dispense different numbers of cells to wells of a 1536-well microplate. By adding different volumes of a cell suspension cell number can be varied, which can be distinguished using the CellTiter-Glo assay. As shown in Figure 5, as few as 200 cells can be accurately and precisely dispensed. This represents a dispense volume of 1 µL. Incremental increases in volume (1 µL) are also significantly different from the 0-cell control as well as the other volumes (Table 2). The signal generated as a function of cell number or dispense volume is linear over the range tested. As seen in Figure 5, the increase in signal is proportional to the increase in cell number.

Linear regression analysis indicates a high correlation of the data.

Cell Number	Mean	STD	Z' factor
0	10	5	-----
200	6446	990	0.54
400	12516	1527	0.63
600	18318	1599	0.74
800	22730	2122	0.72

Table 2. Statistical Comparison of the Luminescent Signal with Different Cell Numbers.

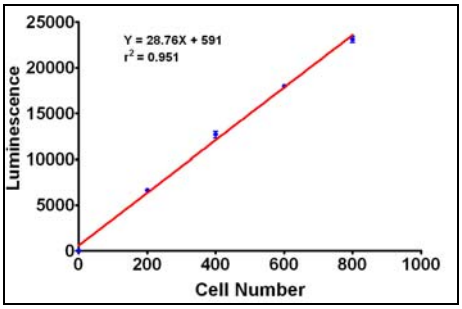

Figure 5. Linearity of Dispense. The MicroFlo Select was used to dispense different volumes of a cell suspension (200 cells/ µL) into 1536-well microplates followed by the addition of media to a final volume of 4 µL. Subsequent to the cell dispense, 4 µL of CellTiter-Glo reagent was added using the MicroFlo Select and the luminescence was determined. Linear regression analysis was then performed on the data.

Discussion

These data demonstrate that the MicroFlo Select can accurately and precisely dispense cell suspensions into 1536-well microplates. The MicroFlo is capable of aliquoting a number of different cell types without loss of viability. While we have utilized the lower volume limit of the dispenser (1 µL) to dispense as few as 200 cells per well, less dilute solutions of cells (e.g. <200 cells/µL) could certainly be utilized if necessary. In our hands CellTiter-Glo assays with 200 cells per well provide a statistically significant signal above background. In addition at this concentration, the MicroFlo is capable of dispensing cell solutions in 1 µL increments with statistically significant differences.

The MicroFlo Select offers many features that allow it to dispense cells into 1536-wel microplates. High resolution plate movement allows the dispenser to position the dispenser tips precisely. Easily removable cassettes can be dedicated for specific solutions, preventing possible cross-contamination. In addition the cassettes are completely autoclavable, which provides an easy way to sterilize the fluid path.

References

[1] Held, P. (2009) BioTek Instruments tech Note. http://www.biotek.com/resources/articles/measure-luminescence-reactions.html