Application Note

Cell Culture QC

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Automated Cell Dispensing into 1,536-Well Microplates for HTS

Using the Agilent BioTek MicroFlo Select to seed tissue culture cells

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Abstract

Today's high-throughput screening (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 1,536-well microplates for cell-based assays requires the use of accurate and reliable automation to dispense uniform numbers of cells to each microplate well in a volume of a few microliters. This application note describes the use of the peristaltic pump-based Agilent BioTek MicroFlo Select to dispense cells into 1,536-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 1,536-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 1,536-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. This application note describes the use of the MicroFlo Select peristaltic pump dispenser to seed 1,536-well microplates with tissue culture cells.

Materials and methods

CellTiter-Glo reagent was purchased from Promega Corp. (Madison, WI). Solid white 1,536-well microplates (part number 3727) and clear bottom, black-sided 1,536-well plates (part number 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 1,536-well microplates using an Agilent BioTek MicroFlo Select. 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 to 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 an Agilent BioTek Synergy 2 multimode microplate reader, 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.

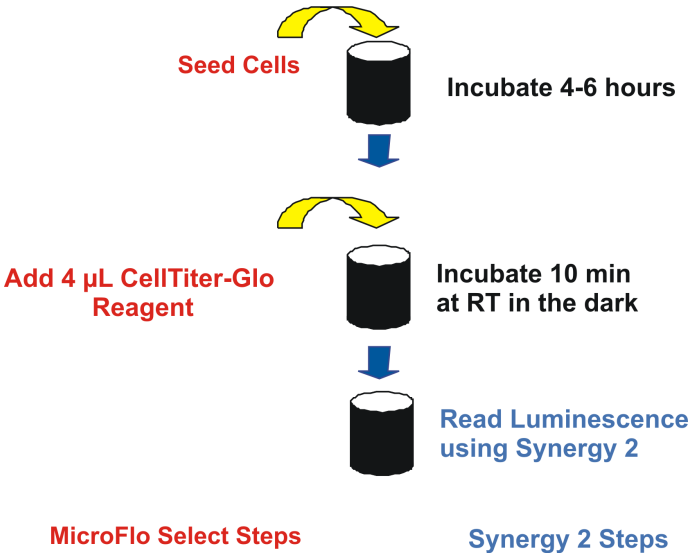

Figure 1. CellTiter-Glo assay procedure.

Results and discussion

Equal numbers of cells (800 cells/well) were dispensed into all the wells of a 1,536-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.

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

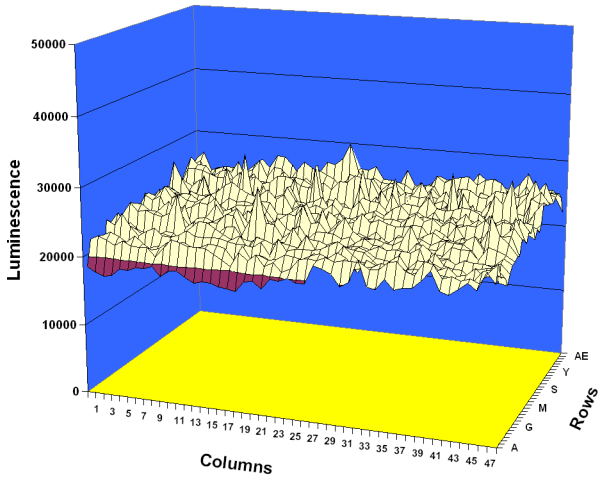

Figure 2. Uniformity of dispensing into 1,536-well microplates. Surface plot of the data generated from a CellTiter-Glo luminescent assay. An Agilent BioTek MicroFlo Select was used to dispense 4 µL of CHO-M1 cell suspension followed by 4 µL of CellTiter-Glo reagent to all the wells of a 1,536-well microplate.

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

Table 1. Whole plate dispense statistics. The mean, standard deviation, and Z' value for CellTiter-Glo data generated from two 1,536-well plates. One plate received 4 µL of media only while the second received 4 µL of cell suspension containing 200 cells/µL.

Cell Number	Mean	Standard Deviation	Z' Factor
0	10	5	–
800	23,210	1,870	0.75

When column and row constancy of a plate is examined, very little difference between either is observed (Figure 3). The MicroFlo Select, 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.

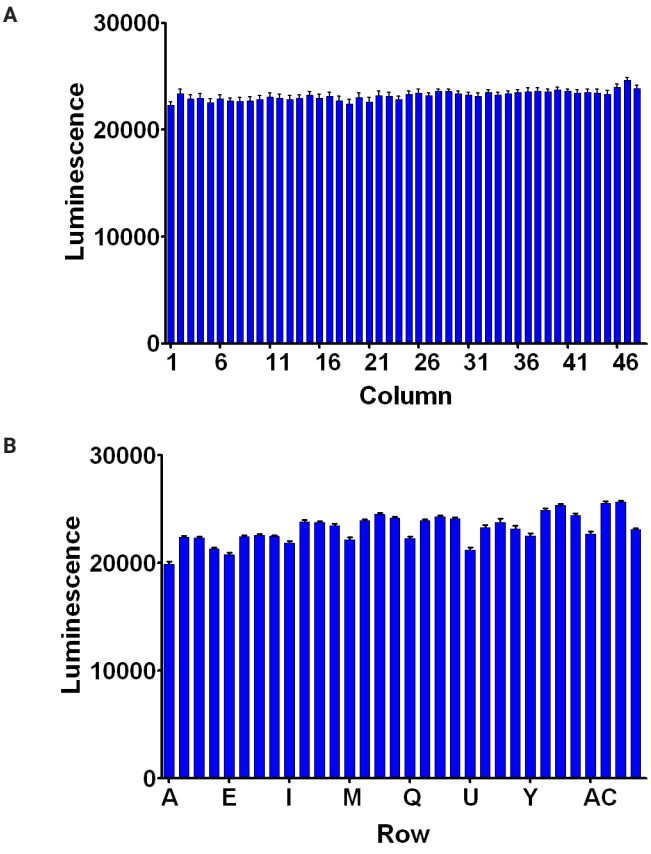

Figure 3. Row and column consistency. The Mean and SEM of each column (A) and row (B) of a 1,536-well CellTiter-Glo assay plate. Note that each data point for column and row consistency represents the mean and SEM for 32 and 48 data points respectively.

When cells are seeded into 1,536-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.

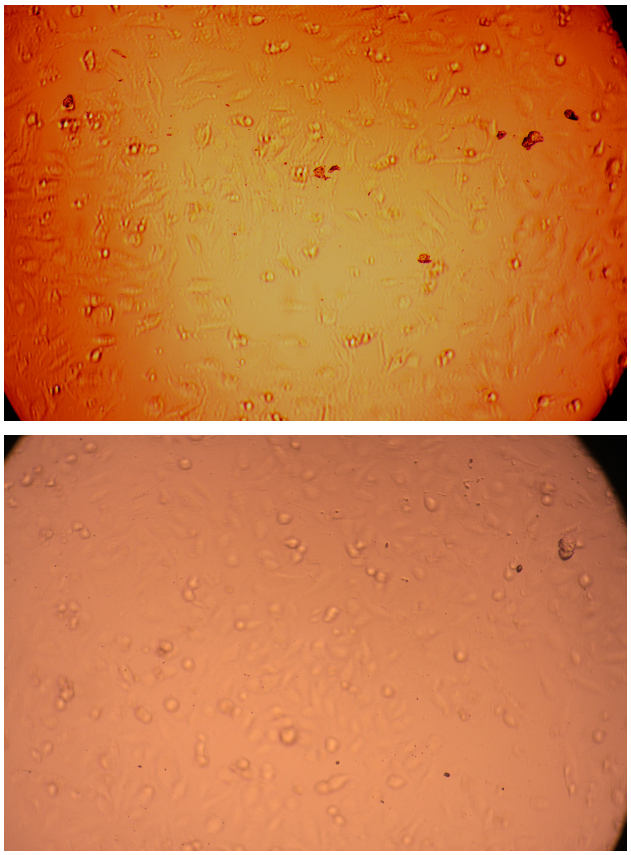

Figure 4. Representative images of h-mesothelioma cells dispensed into 1,536-well plates using an Agilent BioTek MicroFlo Select dispenser. Digital light transmission images were taken with a Zeiss inverted microscope configured with a Nikon camera.

The MicroFlo Select can also be used to dispense different numbers of cells to wells of a 1,536-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.

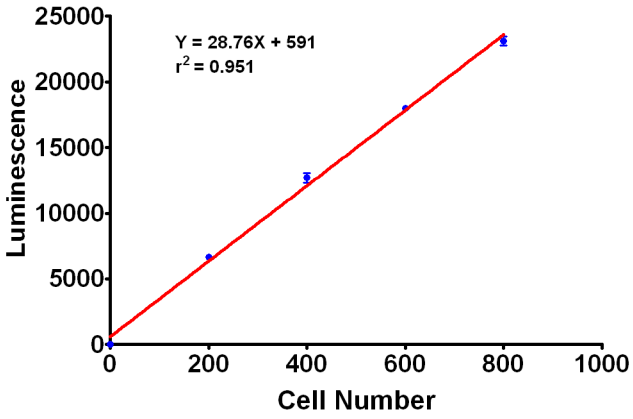

Figure 5. Linearity of dispense. The Agilent BioTek MicroFlo Select dispenser was used to dispense different volumes of a cell suspension (200 cells/µL) into 1,536-well microplates followed by the addition of media to a final volume of 4 µL. Subsequent to the cell dispense, 4 µL of CellTiter-Glo reagent was added using the MicroFlo Select and the luminescence was determined. Linear regression analysis was then performed on the data. Linear regression analysis indicates a high correlation of the data.

Table 2. Statistical comparison of the luminescent signal with different cell numbers.

Cell Number	Mean	Standard Deviation	Z' Factor
0	10	5	–
200	6,446	990	0.54
400	12,516	1,527	0.63
600	18,318	1,599	0.74
800	22,730	2,122	0.72

Conclusion

These data demonstrate that the Agilent BioTek MicroFlo Select dispenser can accurately and precisely dispense cell suspensions into 1,536-well microplates. The MicroFlo Select is capable of aliquoting a number of different cell types without loss of viability. While using 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 be used if necessary. CellTiter-Glo assays with 200 cells per well provide a statistically significant signal above background. In addition, at this concentration, the MicroFlo Select 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 1,536-well 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.

Reference

1. Held, P. *BioTek Instruments tech note*, **2009**. http://www.biotek.com/resources/articles/measure-luminescence-reactions.html.

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