

# Automated Cell Dispensing into 1536-Well Microplates for HTS Screening

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## Abstract

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 performance of the MicroFlo Select peristaltic pump dispenser to dispense different cell lines into 1536-well microplates. As with any cell-based experiment, providing uniform numbers of viable cells is paramount to successful experiments. CHO-M1 cells (200 cells/ $\mu\text{L}$ ) were dispensed into 1536-well microplates (4  $\mu\text{L}$ /well) and cell uniformity as measured by the luminescent determination of ATP using CellTiter-Glo assay kits from Promega. Luminescent signal for wells not containing cells averaged 10, while those wells with 800 cells averaged 23210. The %CV for the entire plate was 8.06% with a  $Z'$  = 0.753 when compared to the plate with media only. The average %CV for the individual rows of the plate was 5.33%, while that of the columns was 7.84%. Details regarding work flow and instrument performance will be provided.

## Assay Process

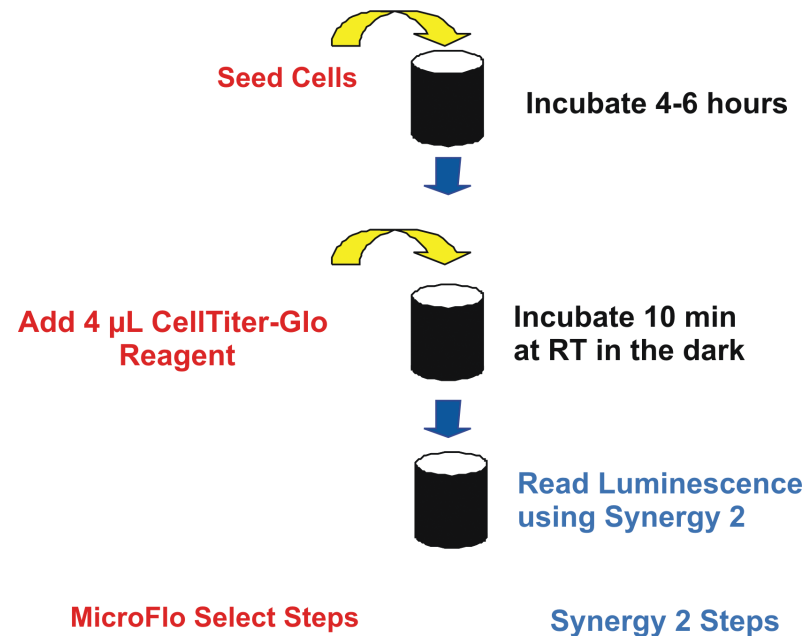


Figure 1 – CellTiter-Glo Assay Procedure

## Uniformity of Dispense

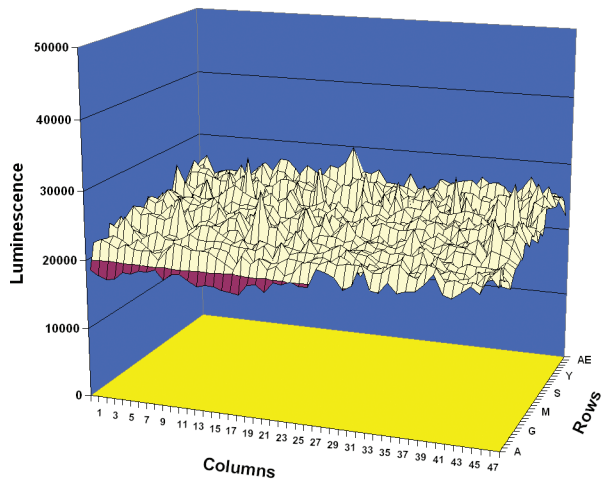


Figure 2 – Uniformity of Dispensing into 1536-well Microplates. Surface plot of the data generated from a CellTiter-Glo luminescent assay. A MicroFlo Select was used to dispense 4  $\mu\text{L}$  of CHO-M1 cell suspension followed by 4  $\mu\text{L}$  of CellTiter-Glo reagent to all the wells of a 1536-well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

Cell Number	Mean	STD	Z' factor
0	10	5	-----
800	23210	1870	0.753

Table 1 –Whole plate Dispense-statistics. The mean, standard deviation and Z' value for Cell Titer Glo data generated from two 1536-well plates. One plate received 4  $\mu\text{L}$  of media only while the second received 4  $\mu\text{L}$  of cell suspension containing 200  $\mu\text{L}/\text{mL}$ .

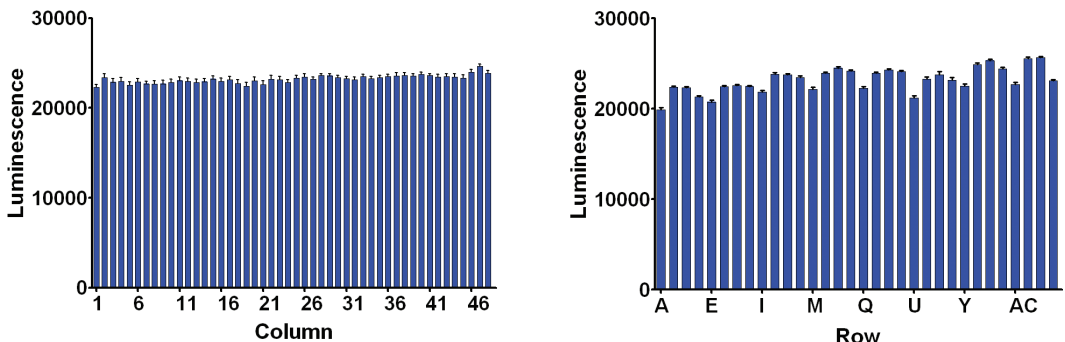


Figure 3 – Row and Column Consistency. The Mean and SEM of each column (A) and row (B) of a 1536-well CellTiter-Glo assay plate. Note that each data point for column and row consistency represents the mean and SEM of 32 and 48 data points respectively.

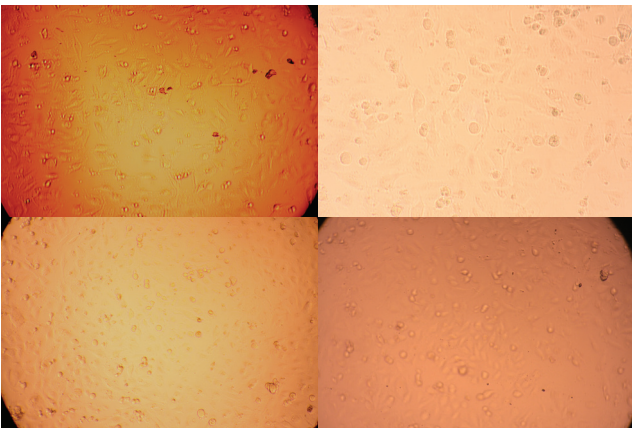


Figure 4 – Representative images of H-mesothelial cells dispensed into 1536-well plates Using a MicroFlo Select. Digital light transmission images were taken with a Zeiss inverted microscope configured with a Nikon camera.

## Incremental Dispense Capabilities

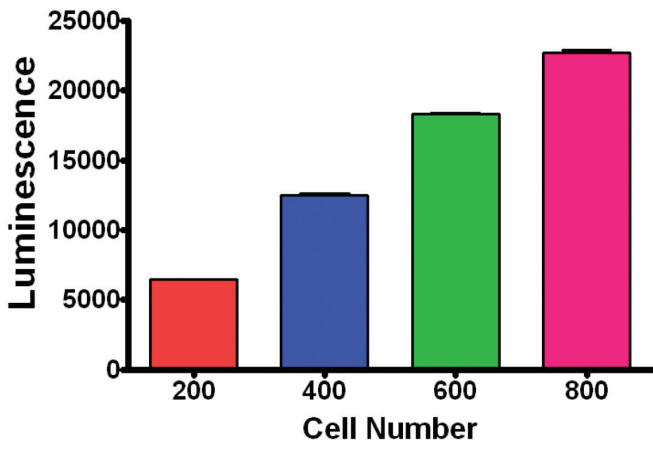


Figure 5 – Luminescence of Different Cell Numbers. Different volumes (1  $\mu\text{L}$ , 2  $\mu\text{L}$ , 3  $\mu\text{L}$ , and 4  $\mu\text{L}$ ) of cell H-mesothelial suspension ( $2 \times 105/\text{mL}$ ) were dispensed into a solid white 1536-well microplate. Cells were allowed to attach for 4 hours after which cell number was assessed using a CellTiter-Glo assay from Promega. Data represents the mean and SEM of 384 determinations.

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

## Linearity of Dispense

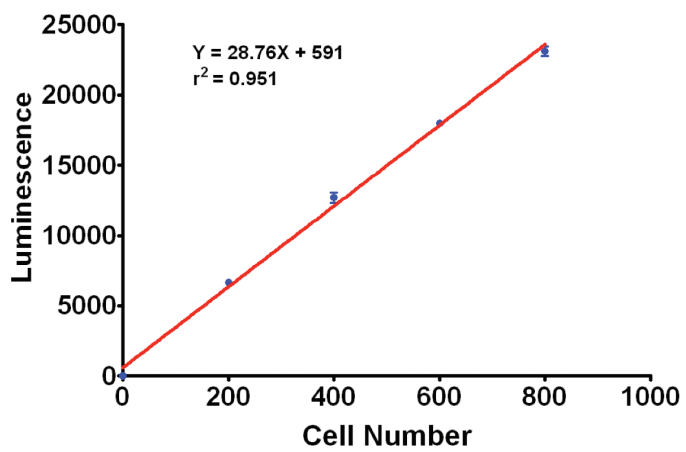


Figure 6 – Linearity of Dispense. The MicroFlo Select was used to dispense different volumes of a cell suspension (200 cells/ $\mu\text{L}$ ) into 1536-well microplates followed by the addition of media to a final volume of 5  $\mu\text{L}$ . Subsequent to the cell dispense, 4  $\mu\text{L}$  of CellTiter-Glo reagent was added using the MicroFlo Select and the luminescence was determined. Linear regression analysis was then performed on the data.

## BIND® Detector Plate Preparation

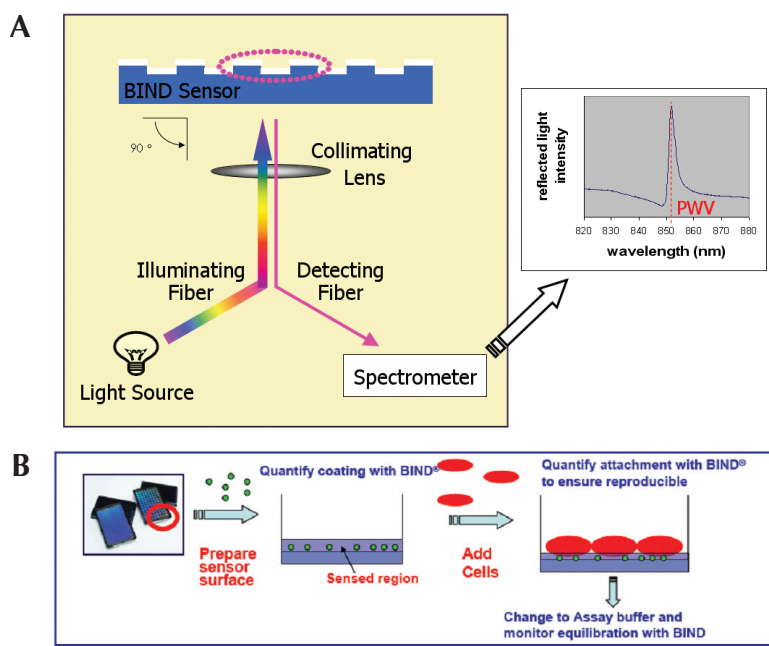


Figure 7 – BIND® technology schematic. (A) BIND sensor detects shifts in the Peak Wavelength Value (PWV) that occur as a result of changes in attached mass. (B) Process of ECM coating and seeding cells to sensor surface.

## Uniformity of Plate Coating and Cell Seeding

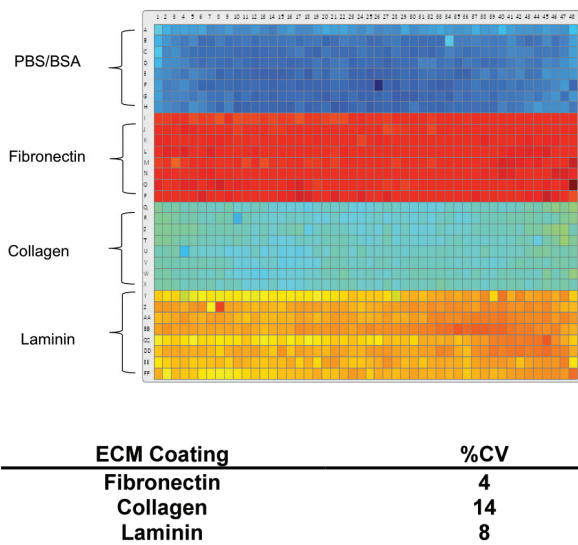


Figure 8 – BIND Assay Heat Map of Different ECM Coatings.

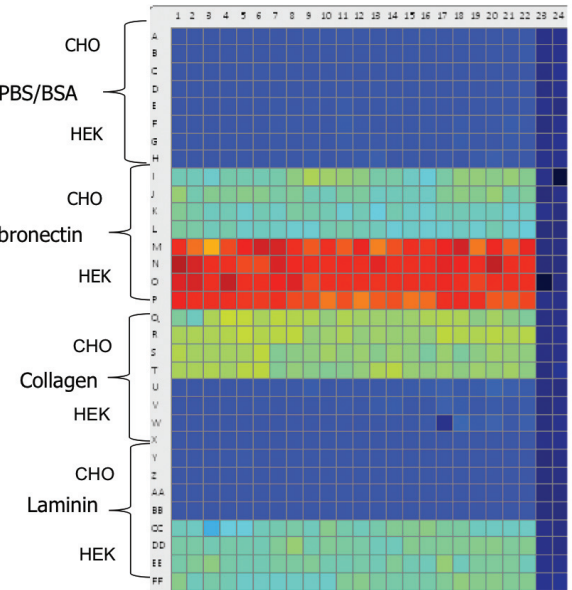


Figure 9 – BIND Assay Heat Map of Cell Dispense. CHO and HEK cells were seeded onto different ECMs previously coated. Note that different cell types prefer different ECM coatings.

## BioTek Instrumentation



Figure 10 – MicroFlo Select Peristaltic Pump Dispenser

The MicroFlo Select offers fast, accurate media and reagent dispensing to different plate types up to 1536-well. It uses a peristaltic pump, with volumes ranging from 1-3000  $\mu\text{L}$ /well, to provide accurate and precise dispenses. The peristaltic pump tubing cassettes come in three different volume ranges (1  $\mu\text{L}$ , 5  $\mu\text{L}$  and 10  $\mu\text{L}$ ), are easily calibrated, installed, and completely autoclavable to provide maximal dispense-accuracy with sterile solutions such as tissue culture media.



Figure 11 – Synergy™ 2 Multi-Mode Microplate Reader

The Synergy™ 2 incorporates both a monochromator system and filters to select wavelengths. The optical head can focus up and down on samples with a 100  $\mu\text{m}$  resolution. It also uses a dedicated optical system, separate from the fluorescence optics, for high-performance luminescence detection. An ultra low noise digital photon integration system and high-quality optics ensure the best sensitivity available today.

## Conclusions

- The MicroFlo Select can accurately and precisely dispense cell suspension into 1536-well microplates.
- Different cell types from different species can be dispensed.
- The MicroFlo Select can dispense as little as 1  $\mu\text{L}$  (200 cells) into the wells of 1536-well microplates with  $Z'$  values greater than 0.5.
- The MicroFlo Select has a 1  $\mu\text{L}$  resolution in regards to dispense-volumes.
- The MicroFlo Select is capable of dispensing the substrate as well as cells for very sensitive assays such as the BIND® technology from SRU Biosystems.
- The fluid path of the MicroFlo Select can be autoclaved make sterilization easy.