# Use of a Flexible Multipurpose Automated 1536-well Microplate Dispenser for HTS

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## MultiFlo™ Dispenser

*Figure 1 – MultiFlo Dispenser.*

## Introduction

Today's HTS demands require that automated dispensers are not only fast and accurate, but also are flexible, compact and serve multiple tasks. Traditional screening technologies employed *in vitro* assays to screen potential drug compounds for activity. However, with advances in HTS screening, drug discovery has migrated toward cell-based assays performed in high-density microplates. 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. As with any cell-based experiment, providing uniform numbers of viable cells is paramount to successful experiments. CHO-M1 cells (200 cells/µL) were dispensed into 1536-well microplates and cell uniformity as measured by the luminescent determination of ATP. Luminescent signal for wells not containing cells averaged 10, while those wells with 800 cells averaged 23210 with a Z' =0.753.

In addition, unique 3-D microcarrier culturing systems such as GEM™ (Global Eukaryotic Microcarrier) have also been automated. These small sub-micron magnetic optically clear particles serve as a carrier for cells avoiding trypsinization prior to plate seeding. We demonstrate the ability of this 3-D cell culture method to measure the activity of the Histamine H1 G-protein-coupled receptor using a FRET-based assay. The entire assay procedure was automated in 384-well format.

The phospho-activation of ERK1/2 can serve as a surrogate marker for G-protein dependent and G-protein independent signals. However, until recently HTS-compatible technologies for measuring ERK activation have been lacking. We have automated a BacMam ERK1/2 Cellular Assay that combines BacMam-mediated gene delivery of a GFP-ERK2 sensor with LanthaScreen® Cellular Assay technology for the measurement of intracellular phospho-ERK1/2 levels. This methodology was used to measure agonist pERK1/2 response at high Z'- factor levels, as well as screen a Tocris library biased towards GPCR and ion channel binding compounds for D2 dopamine receptor antagonists in 384-well microplates.

Here we describe the performance of the MultiFlo dispenser to dispense a number of different reagents under various experimental conditions. Details regarding work flow and instrument performance will be provided as measured by biologically relevant responses in several different experiments.

## Cell Dispensing

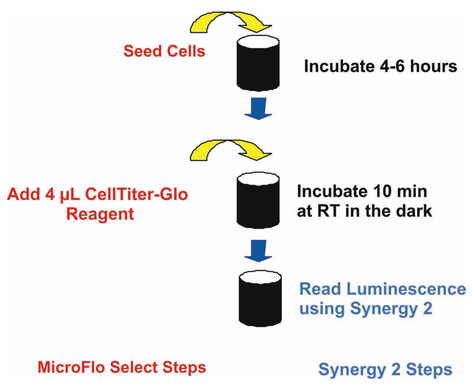



*Figure 2 – Cell dispensing process.*

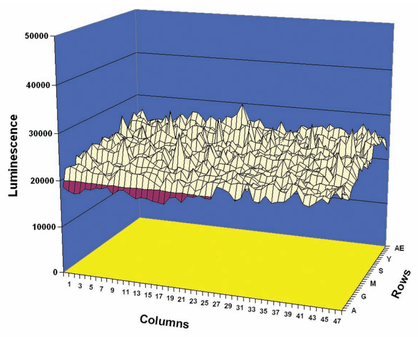



*Figure 3 – Uniformity of dispensing into 1536-well microplates. Surface plot of the data generated from a Cell-Titer Glo luminescent assay. A peripump dispenser module from a MultiFlo was used to dispense 4 µL of CHO-M1 cell suspension followed by 4 µL of Cell-Titer Glo reagent to all the wells of a 1536-well microplate.*

| 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 Cell-Titer 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.*

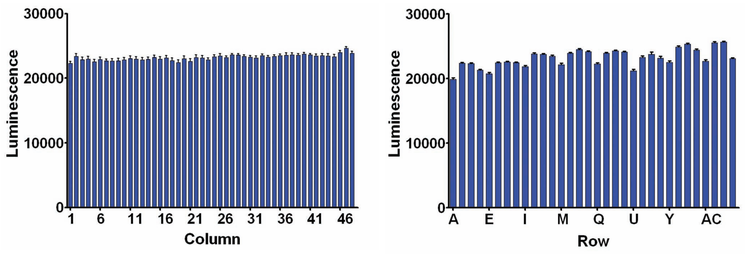



*Figure 4 – Row and column consistency. The mean and SEM of each column (A) and row (B) of a 1536-well Cell-Titer Glo assay plate. Note that each data point for column and row consistency represents the mean and SEM for 32 and 48 data points respectively.*

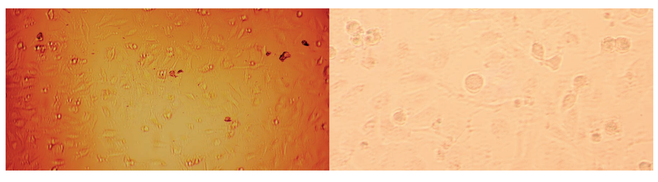
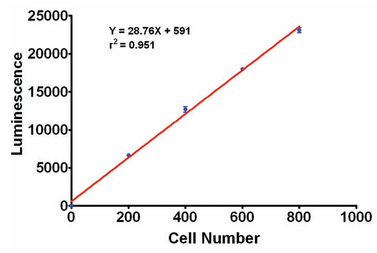


*Figure 5 – Representative images of H-mesothelial cells dispensed into 1536-well plates using a MultiFlo peristaltic pump. Digital light transmission images were taken with a Zeiss inverted microscope configured with a Nikon camera.*

## Cell Dispensing (Continued)




| 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 |

*Figure 6 – Linearity of dispense and results statistics. The MultiFlo peripump 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 Cell-Titer Glo reagent was added using the MultiFlo and the luminescence was determined. Linear regression analysis was then performed on the data. Statistical comparison of the luminescent signal with different cell numbers was also determined.*

## LanthaScreen® Assay

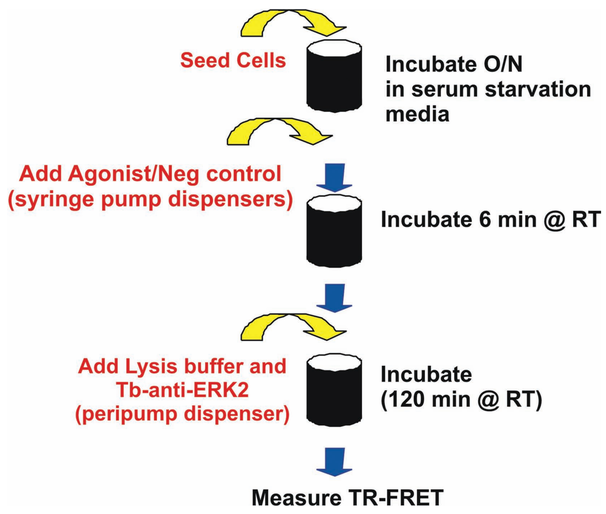
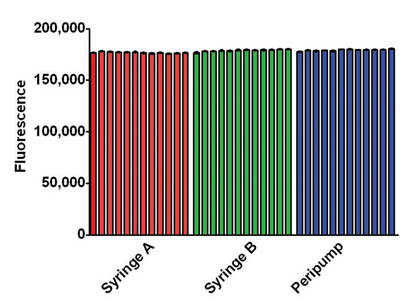



*Figure 7 – Automated LanthaScreen® Assay procedure. A cell line stably expressing a GPCR was transduced with BacMam ERK2 reagent and cryopreserved. The BacMam-treated cells were thawed the day before the experiment, washed in low serum (0.1%) assay media, seeded into 384-well plates (25 µL media/well), and serum starved overnight. Media with or without agonist was added (5 µL) separately using the syringe pump of the MicroFlo and incubated at RT for 6-8 minutes, depending on the GPCR cell line. Cells were then lysed directly (without media aspiration) by the addition of (6 µL) concentrated lysis buffer containing LanthaScreen Tb-anti-ERK2 [pThr185/pTyr187] antibody using the MicroFlo peristaltic pump and TR-FRET signals measured.*

## Uniformity of Dispense




Three different dispenser modules were used to dispense 5 µL of lysis buffer containing fluorescein dye into 192 wells of a 384-well microplate. The fluorescence was determined using a Synergy™ 2 Multi-Mode Microplate Reader. Each data point represents the mean of 16 wells.

*Figure 8 – Dispense Uniformity of Reagent Dispensers.*

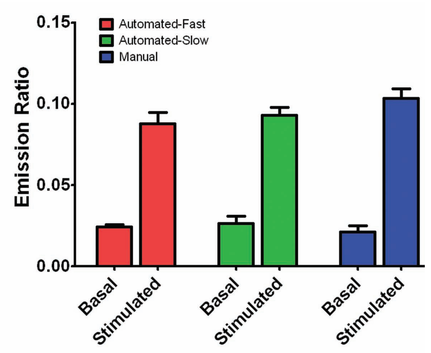



*Figure 9 – Comparison of automated and manual procedures using HTR1A GeneBLAzer® CHO cells that were transduced with BacMam ERK2 reagent.*

| | MultiFlo Disp | | |
|---|---|---|---|
| | Fast Dispense | Slow Dispense | Manual Dispense |
| Z' factor | 0.62 | 0.57 | 0.64 |
| Response Ratio | 3.62 | 3.51 | 4.84 |

*Table 2 – Statistical comparison between automated and manual liquid handling.*

## ERK2 Response to EGF stimulation

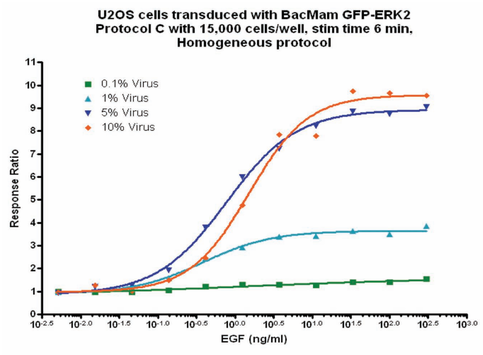



*Figure 10 – U2OS cells transduced with BacMam ERK2 virus at four different concentrations. The following day cells were stimulated with EGF for 6 minutes followed by lysis with Cellular LanthaScreen® Lysis buffer containing Tb-anti-phospho-ERK2 antibody. TR-FRET signal was determined and data normalized to the response ratio.*

## D2 Dopamine Receptor Antagonist ERK2 Screening

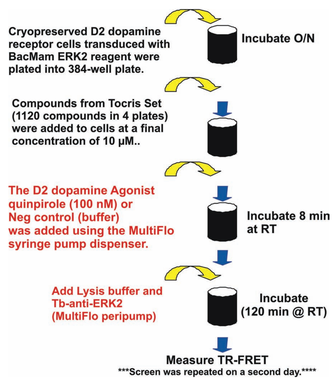



*Figure 11 – D2 dopamine antagonist screen procedure*

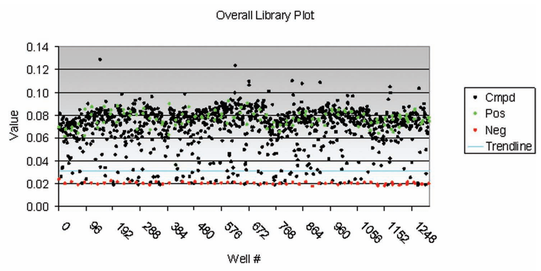



*Figure 12 – Library screening plot. Phospho-ERK2 values from a single screen, with cutoff value indicated. Eventual results indicating multiday inhibitors are depicted as red data points.*

| Day 1 | |
|---|---|
| Plate | Z' |
| 1 | 0.61 |
| 2 | 0.53 |
| 3 | 0.59 |
| 4 | 0.61 |

- 67/1120 Compounds scored as inhibitors in preliminary screen
- All D2 antagonists were detected

| Follow–up Screen | |
|---|---|
| Plate | Z' |
| 1 | 0.50 |
| 2 | 0.65 |
| 3 | 0.63 |
| 4 | 0.70 |

- 55/1120 compounds were inhibitors on both days
- 20/55 were reported dopamine receptor inhibitors
- 4/55 were reported MEK inhibitors
- All D2 antagonists were detected

*Table 3 – Screening statistics. Compounds which showed inhibitory activity below the cutoff on both days were scored as "hits". For each screen, cutoff was set at 6% (67/1120 compounds). The Tocris library is biased towards GPCRs and ion channels, so this hit rate is reasonable.*

## GEM seeding and GeneBLAzer Histamine Assay

The GEM is a novel alginate three-dimensional cell microcarrier with a chemical structure similar to components of the extracellular matrix and provides a unique porous surface that mimics *in vivo*-like metabolic function. The GEM is non-autofluorescent and optically clear, allowing for cell assay directly on the substrate, and also contains paramagnetic particles allowing for easy manipulation of cells on the microcarrier.

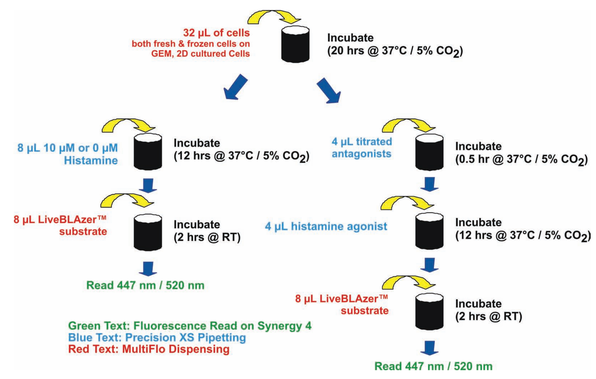
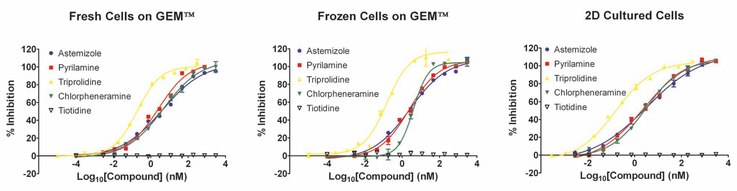



*Figure 13 – Automated GeneBLAzer Histamine H1 assay procedure*

GeneBLAzer® H1-NFAT-*bla* HEK 293T cells contain the human Histamine Subtype 1 receptor (H1), stably integrated into the genome and a β-lactamase (*bla*) reporter gene under control of the NFAT response element. Stimulation or inhibition of the Histamine H1 receptor will be relayed through the NFAT pathway, causing increased or decreased production of β-lactamase, respectively. Addition of the β-lactamase substrate to a well lacking *bla* results in the substrate remaining intact. Upon excitation at 409 nm, energy is transferred from coumarin to fluorescein, and emitted at 520 nm. After addition of the β-lactamase substrate to a well containing *bla*, the substrate is then cleaved. Upon excitation at 409 nm, FRET does not take place, and emission is seen at 447 nm.




**Antagonist IC50 Values (nM)**

| | Astemizole | Pyrilamine | Triprolidine | Chlorpheneramine |
|---|---|---|---|---|
| Fresh Cells on GEM™ | 4.197 | 2.603 | 0.1859 | 4.563 |
| Frozen Cells on GEM™ | 2.613 | 2.480 | 0.1546 | 4.012 |
| 2D Cultured Cells | 3.278 | 2.309 | 0.1559 | 3.064 |
| Reported $IC_{50}$ Value | 4.0 | 4.9 | 0.098 | 9.2 |

*Figure 14 – Histamine H2 antagonist curves and $IC_{50}$ values*

## Conclusions

- The MultiFlo can accurately and precisely dispense cell suspensions into 1536-well microplates.
- The MultiFlo can dispense as little as 1 µL (200 cells) into the wells of 1536-well microplates with Z' values greater that 0.5.
- The MultiFlo has a 1 µL resolution in regards to dispense volumes.
- The MultiFlo dispensers are capable of providing equivalent amounts of LanthaScreen reagent.
- Use of the MultiFlo Dispenser for the ERK2 cellular LanthaScreen assay produced lower errors than manually performing the assay, in a side-by-side experiment.
- Use of the MultiFlo Dispenser for an ERK2 cellular LanthaScreen assay produces a slightly lower response ratio than when the assay was performed manually, but Z' values were comparable through improved precision.
- The MultiFlo Dispenser is capable of automating the addition of agonist and the addition of a LanthaScreen cellular lysis buffer containing Tb-labeled-antibody.
- Biologically focused compound libraries can be easily and accurately screened for D2 antagonist activity using the MultiFlo dispenser in combination with the LanthaScreen cellular phospho-ERK2 assay.
- Cells on the GEM substrate are able to be dispensed consistently across a 384-well plate using the MultiFlo Dispenser.
- The combination of the MultiFlo, the GEM 3D culturing substrate and GeneBLAzer cell-based assays provide a easy-to use robust automated cell-based assay solution for drug discovery.

*GeneBLAzer and LanthaScreen are registered trademarks of Invitrogen Corporation.*