

Overview

- The dispensing capabilities of the peristaltic pump on the EL406 make it ideal for sterile dispensing of tissue culture cells, including primary cells.
- The ability to aspirate media using the wash manifold allows the instrument to perform rapid media exchanges without disturbing delicate cell monolayers.
- The additional dispensing capabilities of the EL406 makes it possible to use the instrument for multiple dispense steps in a single assay procedure.
- The small footprint and sterilization capabilities of the EL406 allow for sterile processing of cells, media, and other components during multi-day assay procedures.
- By combining the instrument with the Precision Microplate Pipetting System and Synergy Microplate Readers, cell-based assay procedures can be automated in a manner that is easy to use, and delivers robust data that is equal to or of higher quality than manually processed samples.

Introduction

Today's pharmaceutical and biotech industry has seen an increased emphasis on generating the most relevant data possible in the most efficient manner. This has meant that many portions of the drug discovery process which typically implemented biochemical assays are now using cell-based assays with increased regularity. This is due to the fact that using intact cells provides a more *in vivo*-like environment when compared to biochemical assays, which use purified enzymes. This has created the need for instrumentation that is capable of handling cells and other assay components in a sterile manner. Large dispensing systems can be difficult to use with this type of assay due to their need for containment systems that can be expensive and difficult to implement. A smaller system, with multiple dispensing capabilities, which does not require its own containment, can deliver the accuracy, flexibility, and ease of use that is necessary for these assay processes.

Here we show the utility of a combination washer dispenser to be used in a cell-based assay format. The small footprint of the instrument allows it to be easily inserted into existing laboratory laminar flow hoods. The accuracy of the peristaltic pump, combined with the ability to autoclave the dispensing cassette allow for sterile dispensing of cells, media, and other assay components. The instrument was used in combination with microplate pipetting and detection systems for automated sample processing. Testing was then performed with three cell-based assay processes representing varied areas of drug discovery research. The first was a triplex assay to measure Cytochrome P450 induction as well as cell viability. The second was a drug absorption assay to measure permeability and active transport of compounds across a cell layer using MDCKII-MDR1 and Caco-2 cells. The final procedure examined the ability to monitor the VEGF signaling pathway within a cell, leading to AKT phosphorylation. Validation data demonstrates the ability of the instrument to dispense cells, perform media exchanges, and deliver assay components to cell and assay plates. Further testing illustrates the ability of the complete system to be used for the increasing number of cell-based assays being implemented in today's life science research laboratory.

EL406 Combination Washer Dispenser



Figure 1 – EL406 Combination Washer Dispenser

The EL406 combines full plate washing capabilities along with three reagent dispensers in a single instrument. The unit has a small footprint, making for easy insertion into existing laminar flow hoods. This allows for sterile manipulation of cells, media, and other reagents.

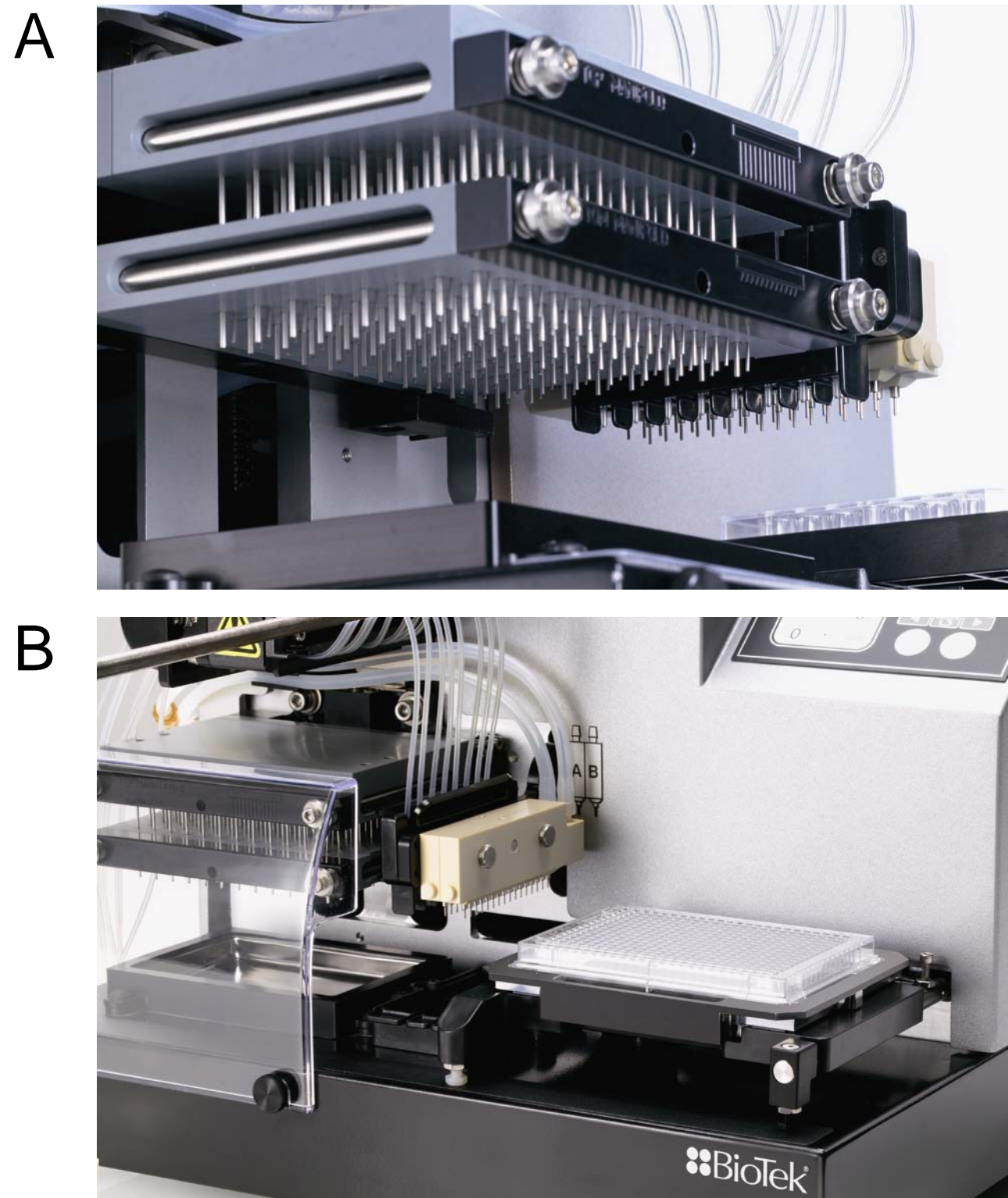


Figure 2 – EL406 Washing/Dispensing Capabilities. **A.** 96-tube Plate Washer; **B.** Peristaltic and Syringe Dispensing Systems

The wash manifold can be used for aspiration of media or buffer during media exchanges. Fine tuning of the Z height allows for media removal without pulling cells off the bottom of the well.

The peristaltic and syringe pump can be used for the dispensing of cells, media, and other assay reagents. Autoclaving and chemical sterilization allow for sterile dispensing of components during the assay process.

Additional Instrumentation for Cell-Based Assay Performance

Precision Microplate Pipetting System

The Precision™ combines an 8-channel pipetting head and an 8-channel bulk reagent dispenser in one unit. The instrument can be used to serially titrate compounds and transfer them, as well as other assay components, into 96- or 384-well assay plates. The small footprint allows for easy insertion into laminar flow hoods. The use of pre-sterilized disposable tips creates the ability for sterile component transfers.

Synergy Hybrid Multi-Mode Microplate Readers

The Synergy™ line of microplate readers combine a filter-based and monochromator-based detection system in the same unit. The readers can be used to detect luminescence, fluorescence, or absorbance based signals from assay plates.

Triplexed Cytochrome P450 Induction/Viability Assay using Primary Hepatocytes

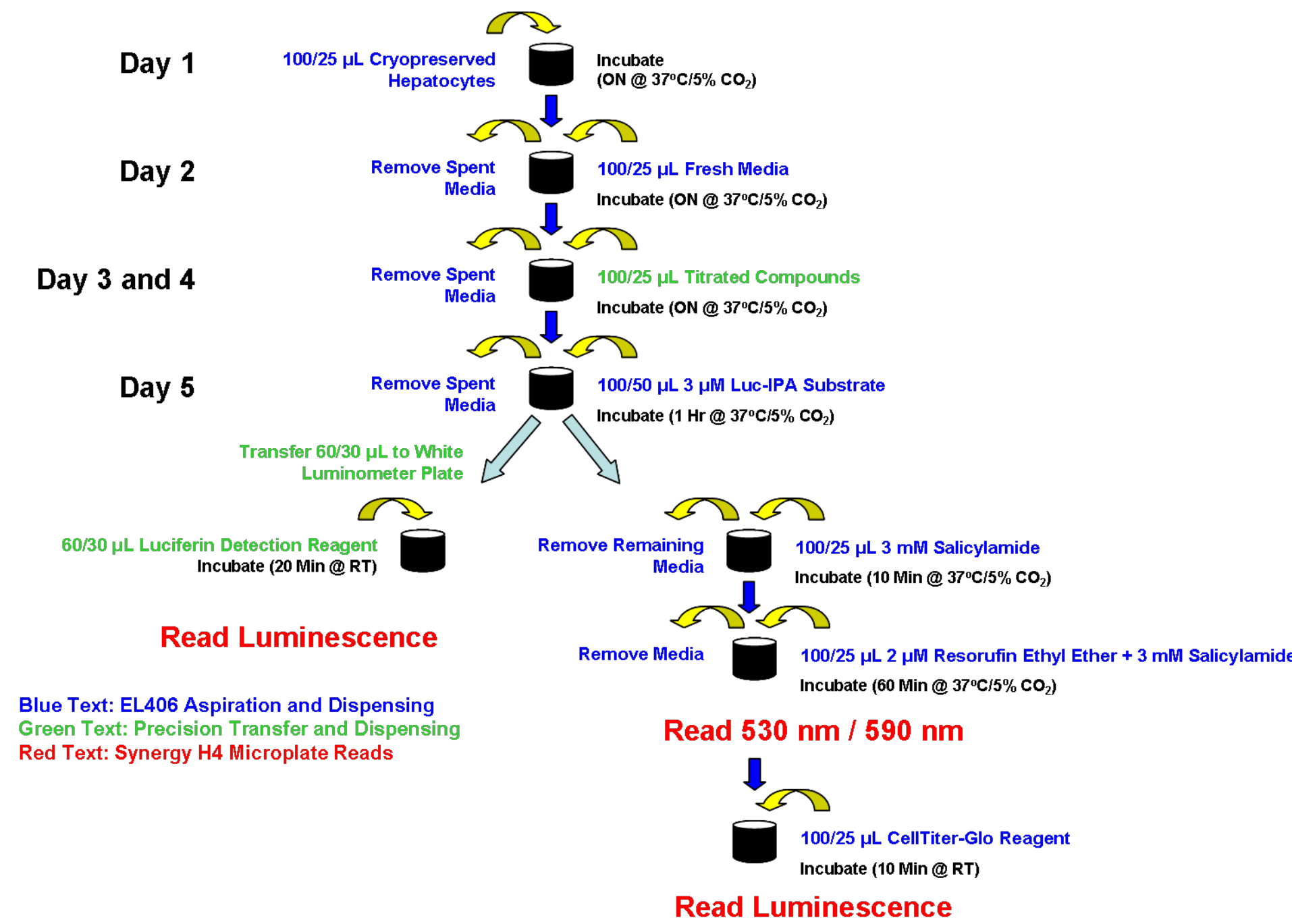


Figure 3 – Triplex assay workflow. EL406 is responsible for cell dispensing, media removal and exchanges, and component additions throughout the assay procedure.

Hepatocyte Plating

The ability of the EL406 to accurately dispense hepatocytes to 96- and 384-well plates was verified by analyzing the %CV of CellTiter-Glo® values across all wells to contain cells in the assay (96-well: Columns 1-11; 384-well: Columns 1-22). 50,000 cells/well, and 10,000 cells/well were dispensed in 96- and 384-well formats, respectively. %CV values were 4.37% for 96-well dispensing, and 5.68% for 384-well dispensing.

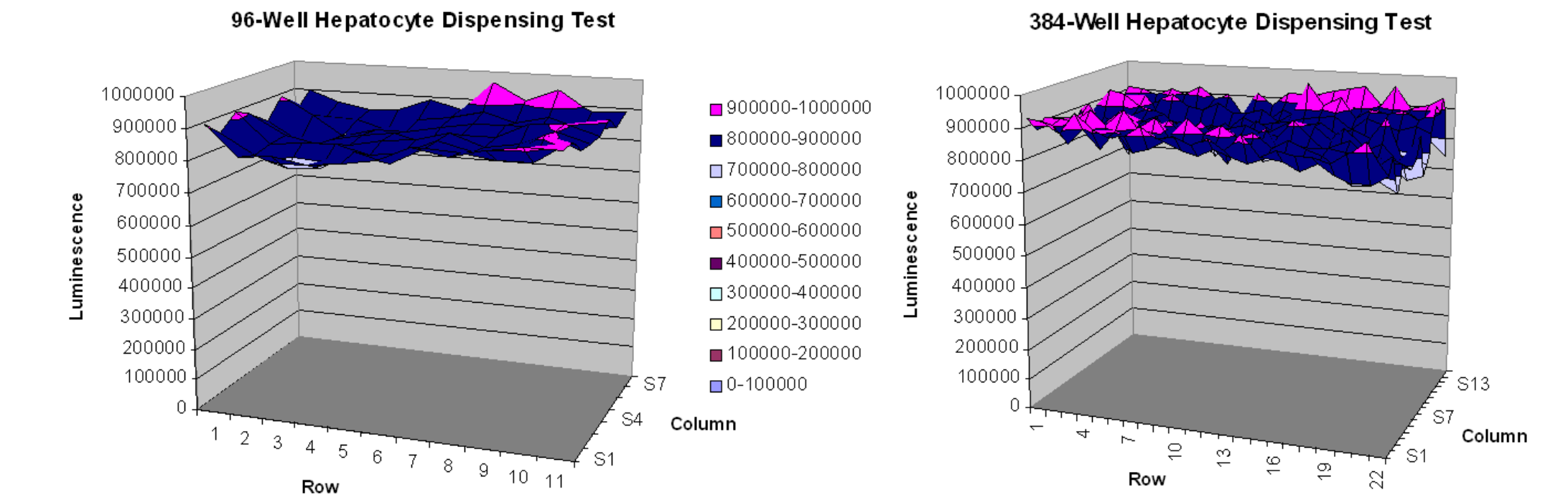


Figure 4 – Plating efficiency of hepatocytes by EL406

Z'-Factor Validation

Z'-Factor assays were performed to validate the fully automated CYP1A and -3A assays. Omeprazole was used as the control inducer for the CYP1A assay, while Rifampicin was used as the control inducer for the CYP3A assay. Forty-eight replicates of 10 µM or 0 µM compound were used as the positive and negative control, respectively.

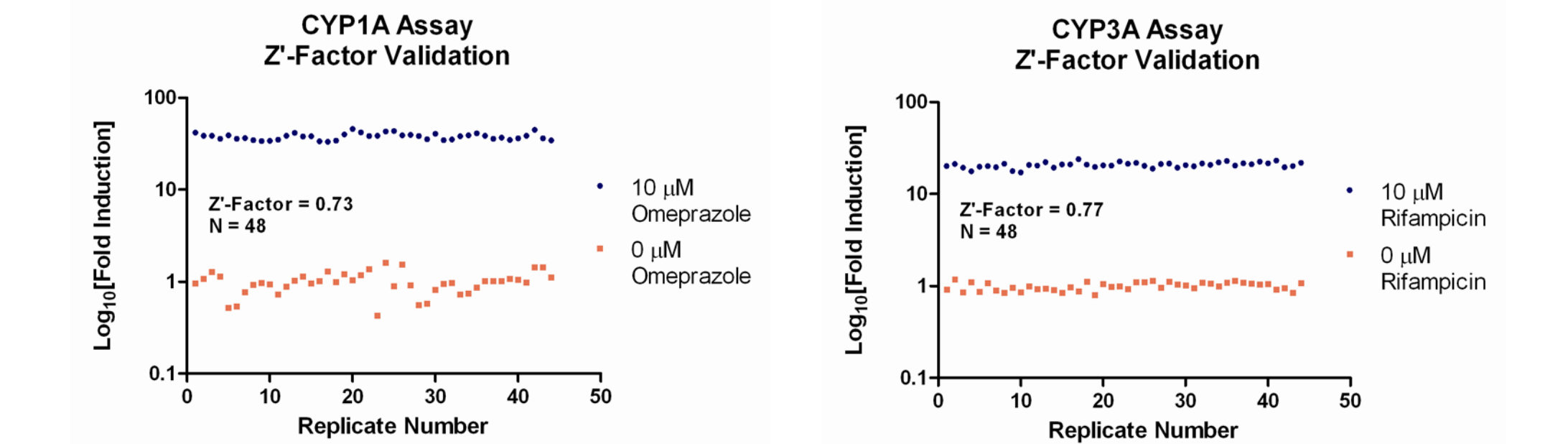


Figure 5 – Z'-Factor Validation Data. Z'-Factor values above 0.5 indicate that each is an excellent assay¹.

Compound EC50 Validation

EC50 values for the control compounds Omeprazole and Rifampicin were computed to further validate the assay. Three runs from a single hepatocyte donor were performed to ensure data consistency. The assay was also performed in 96-well format as an additional control for the 384-well data. The EC50 values for each run fell within the average +/- 1/2 log concentration range of 2-21 µM for Omeprazole and 0.5-5.0 µM for Rifampicin, demonstrating the consistency of the automated assay procedure.

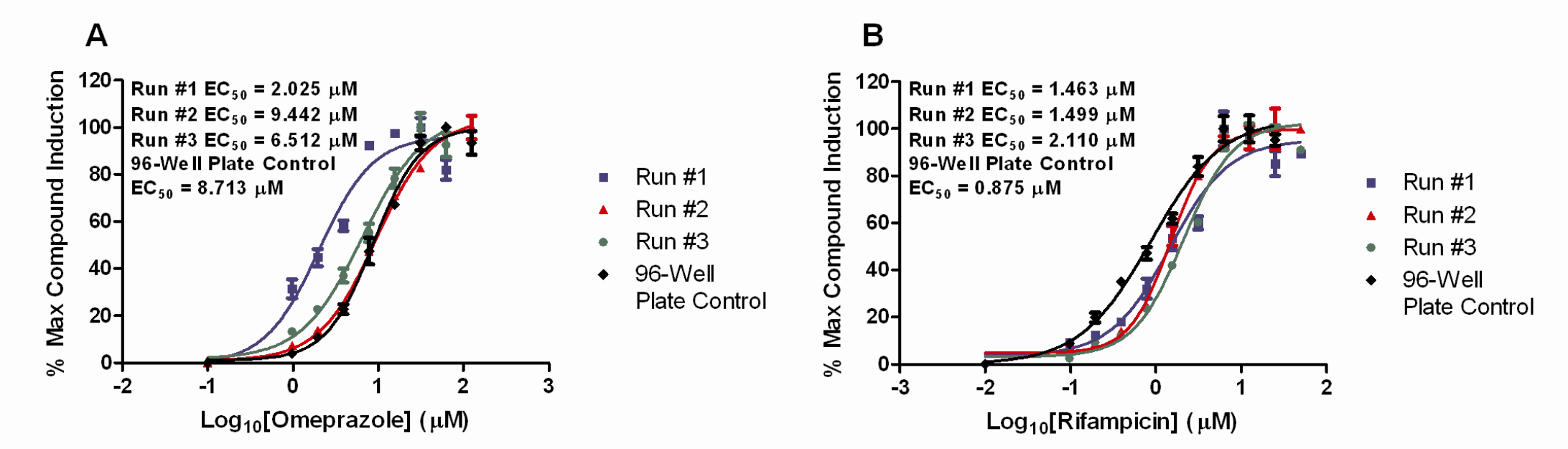


Figure 6 – Omeprazole and Rifampicin multi run and multi donor EC50 curves

Drug Absorption Assays using MDCKII-MDR1 or Caco-2 Cells

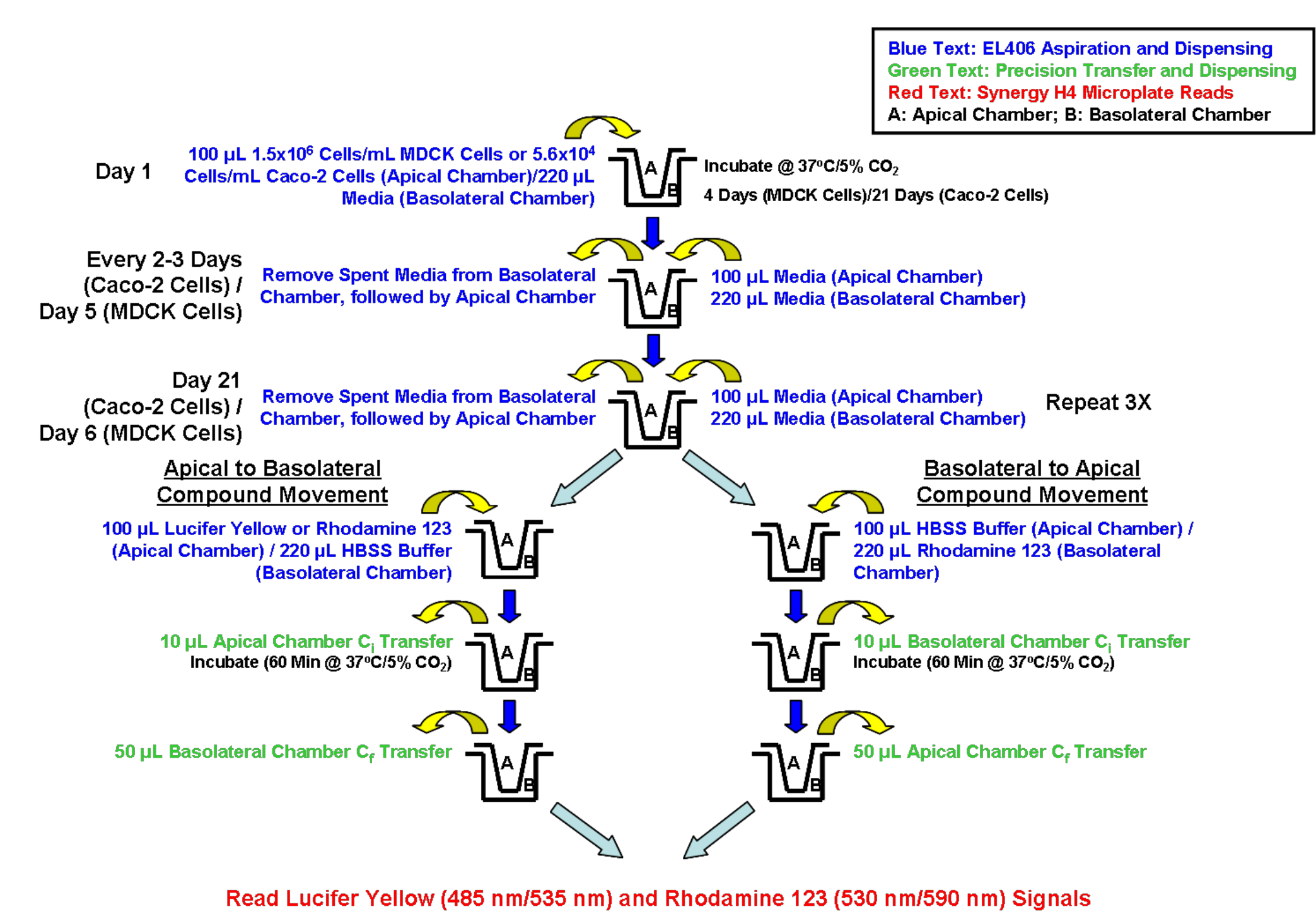


Figure 7 – Automated Drug Absorption assay workflow. EL406 is responsible for cell dispensing, media removal and exchanges to both the apical and basolateral chambers. The instrument is also responsible for buffer fluorescent compound additions to test the automated procedure.

Cell Monolayer Integrity Validation

Papp values for Lucifer Yellow were significantly lower, and showed less variation, indicating a tighter, more consistent cell monolayer for plates processed using the EL406, as compared to manually processed plates.

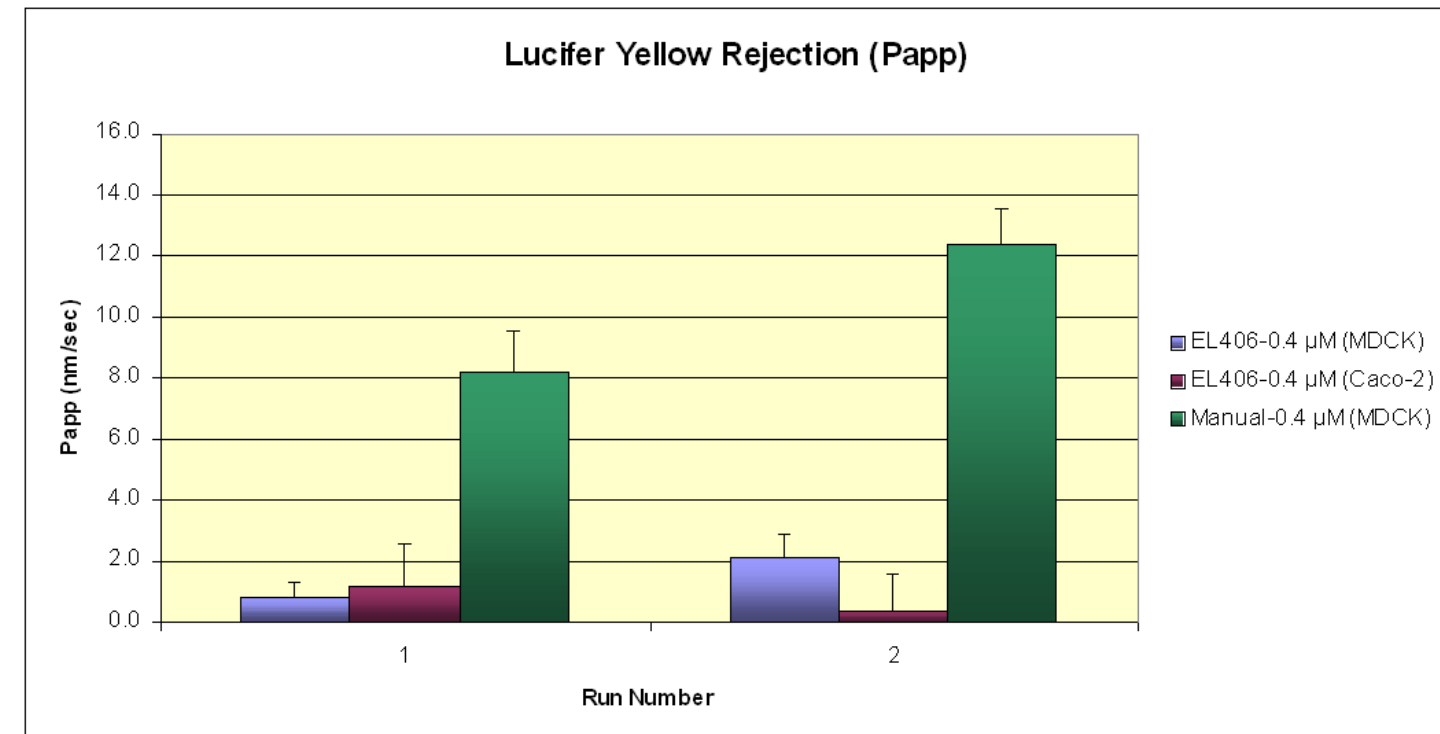


Figure 8 – Average apparent permeability values (Papp) for Lucifer Yellow across two runs for 0.4 µM Transwell® permeable supports processed using EL406 or manual method (n=40). (Data for 1.0 µM Transwell® permeable supports not shown.)

Drug Transport Analysis

Lower Rhodamine 123 apical-basolateral (A-B) values and higher basolateral-apical (B-A) values for EL406 processed plates demonstrate a more intact cell layer and higher functioning P-glycoprotein, respectively, when compared to manually processed plates. Further tests with Caco-2 cells show decreases in efflux values in wells containing Cyclosporin A, a known inhibitor of P-glycoprotein. These results indicate that inhibitor studies are able to be carried out using the automated method.

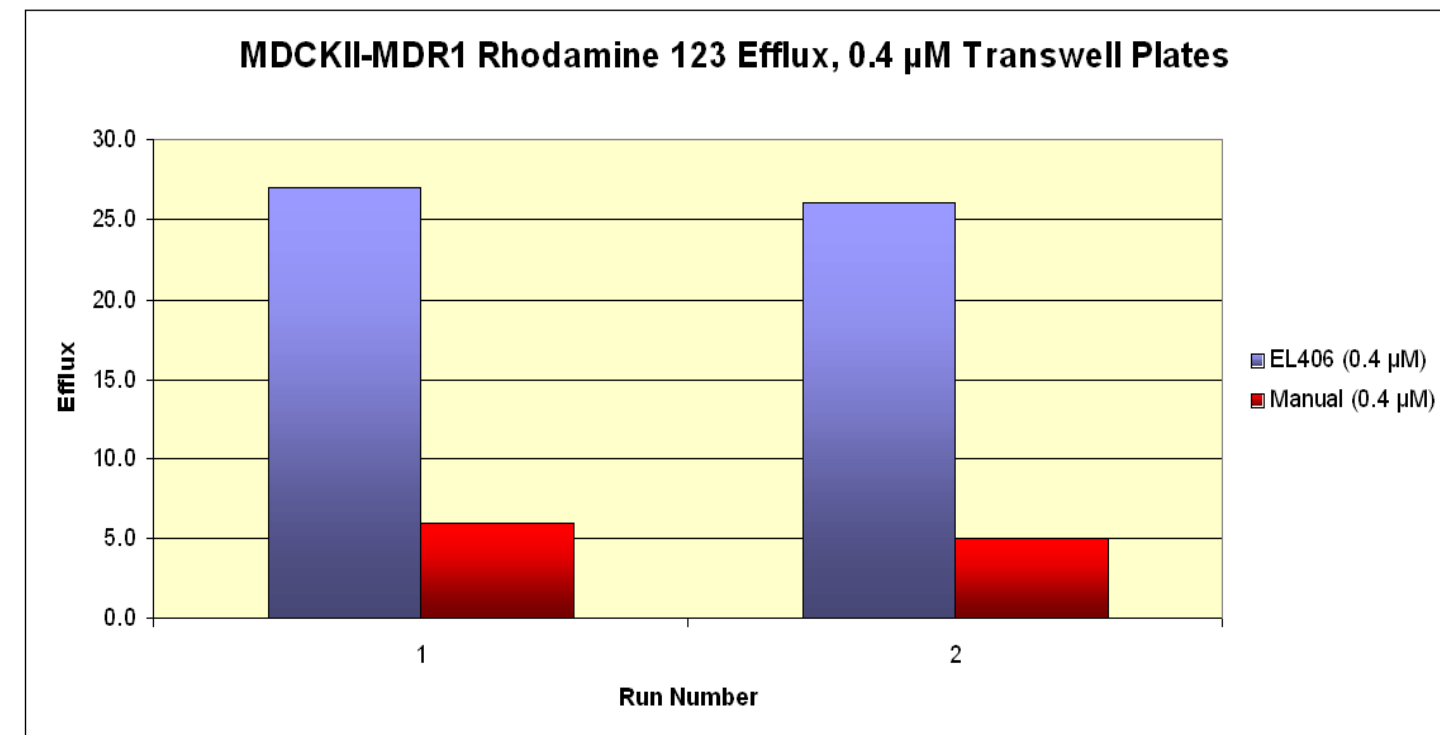


Figure 9 – Average calculated Efflux values for Rhodamine 123 across two runs of 0.4 µM Transwell® permeable supports processed using EL406 or manual method (n=40). (Data for 1.0 µM Transwell® permeable supports not shown.)

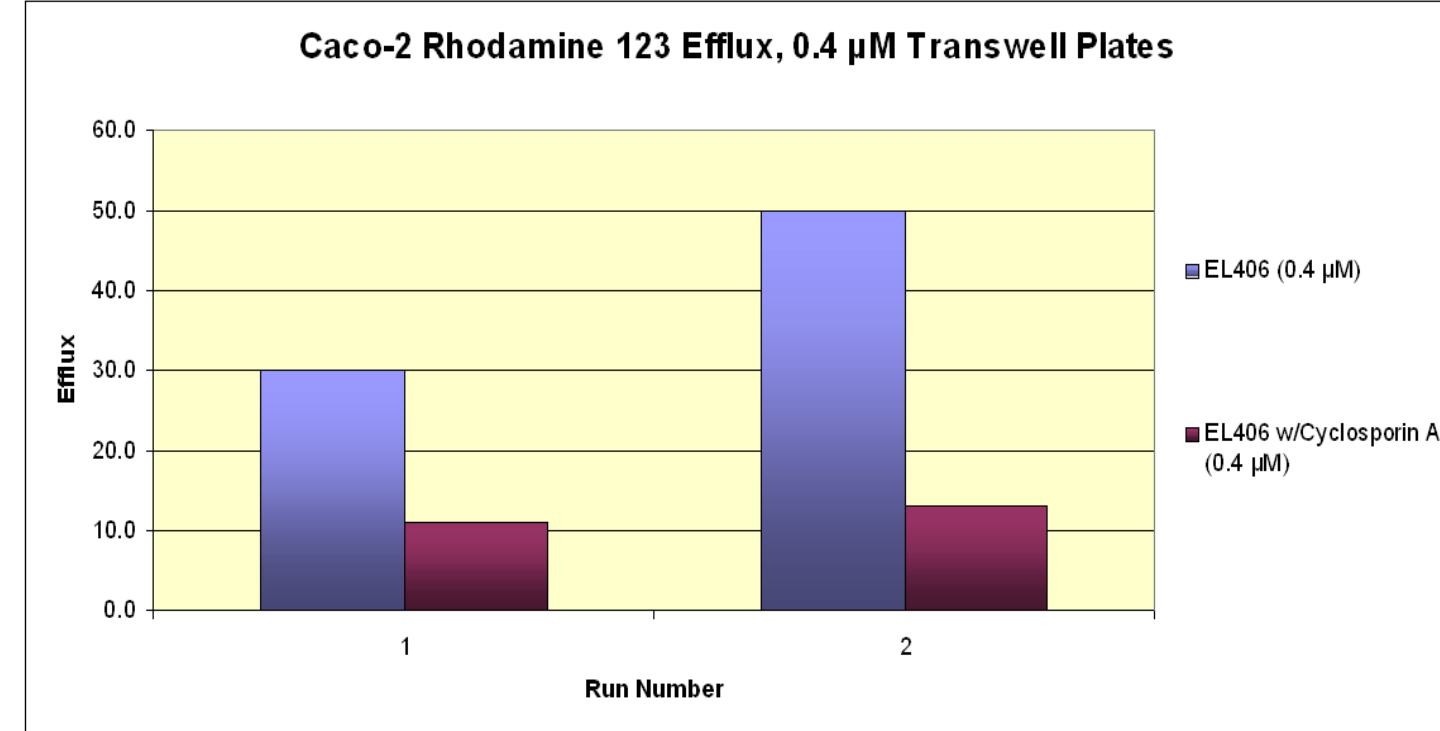


Figure 10 – Average efflux values for uninhibited and inhibited Rhodamine 123 across two runs of 0.4 µM Transwell® permeable supports processed using EL406 (n=40). Uninhibited apical wells for B-A transfer contained HBSS buffer w/1% DMSO, while inhibited apical wells contained 10 µM Cyclosporin A in HBSS buffer. (Data for 1.0 µM Transwell® permeable supports not shown.)

PI3K-AKT-mTOR Signal Transduction Assay using Primary HUVEC Cells

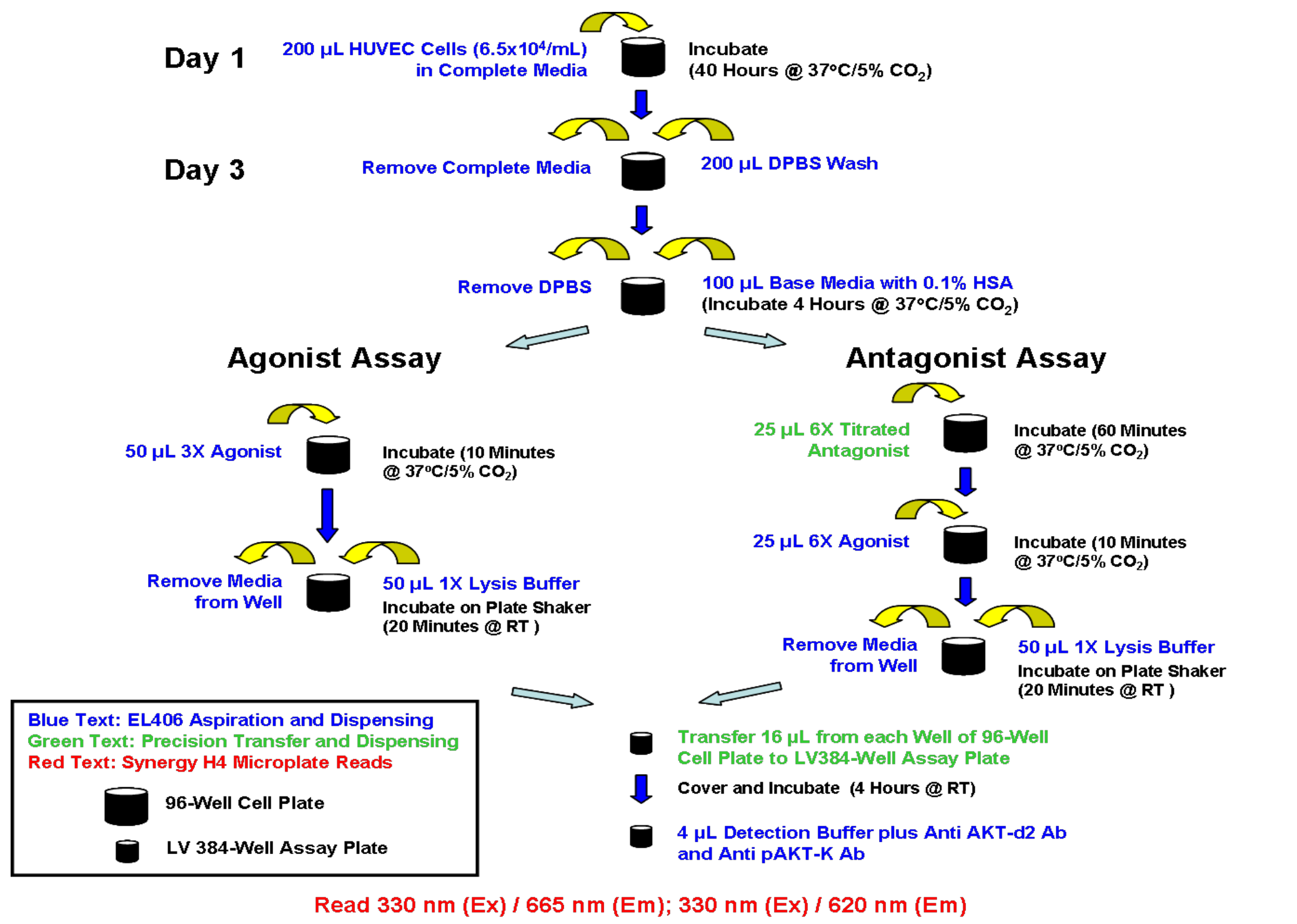


Figure 11 – Automated HTRF® AKT assay workflow. EL406 is responsible for cell dispensing, media removal and exchanges, EC80 agonist and antibody additions.

HUVEC Plating

The ability of the EL406 to accurately dispense HUVEC cells to the 96-well cell plates was verified by analyzing the %CV of CellTiter-Glo® values across test wells containing cells. 13,000 cells/well were dispensed to columns 1, 4, 7, 10, and 12. The average luminescent values for each column were equivalent, while the %CV value across all test wells was 4.87%.

Z'-Factor Validation

A Z'-Factor assay was performed to validate the fully automated HTRF® AKT assay. PI-103, a known PI3 kinase inhibitor, was added to half of the wells, while media was added to the other half. VEGF was then added to each test well.

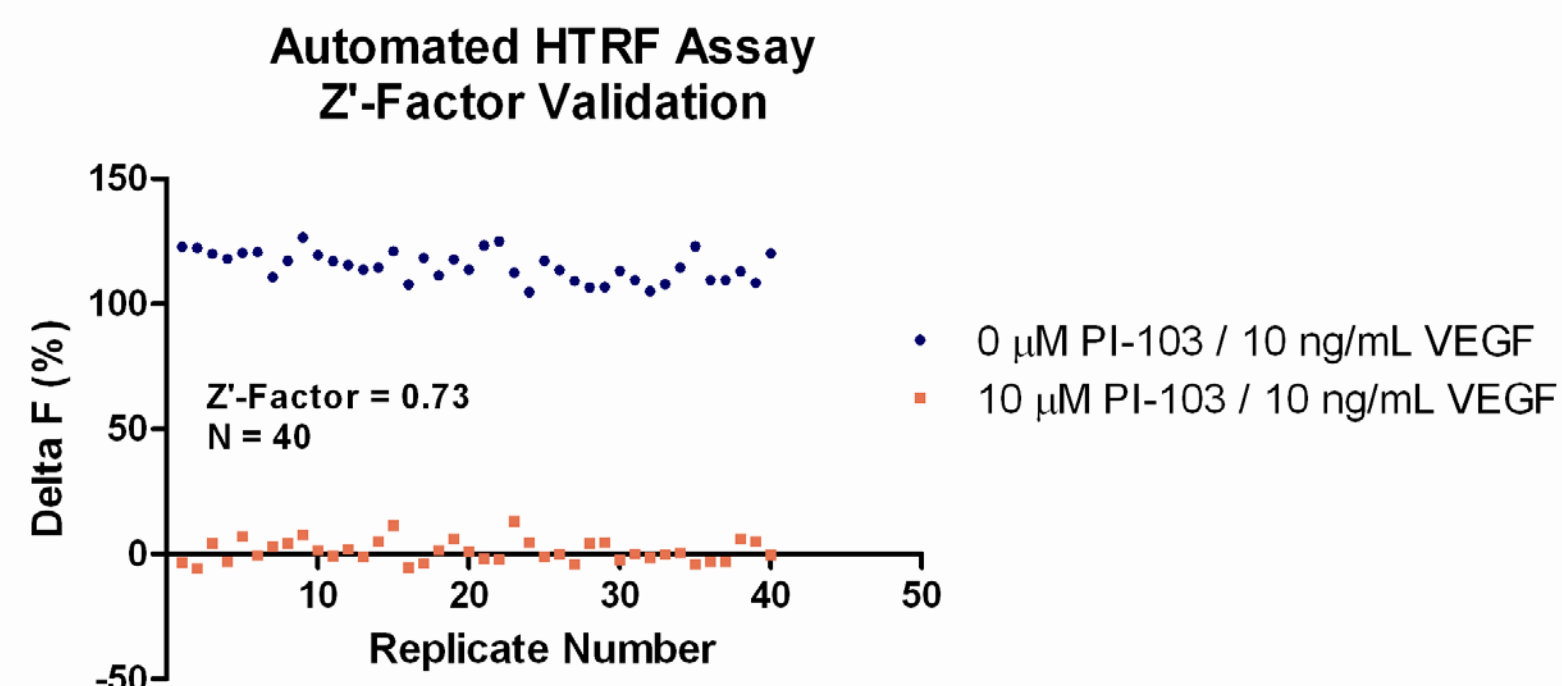


Figure 12 – Z'-Factor Validation Data.

Assay Pharmacology Validation

The assay was further validated by creating a dose response curve with VEGF. Concentrations tested ranged from 40 – 0 ng/mL. The EC50 value generated was 2.1 ng/mL. This value compares favorably to literature EC50 value ranges of 1-6 ng/mL². The assay was run in manual format. Further testing of the automated assay will include pharmacology testing of agonists and antagonists.

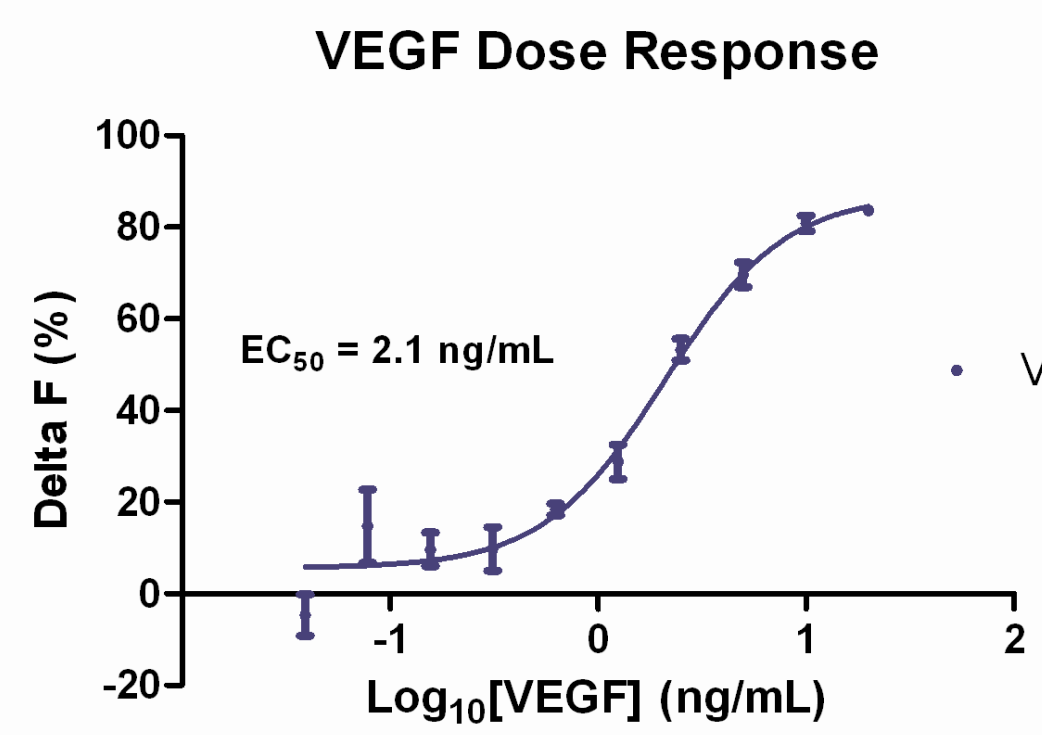


Figure 13 – VEGF EC50 curve

Conclusions

- EL406 is capable of evenly dispensing tissue culture cells, including primary cells, such as hepatocytes and HUVECs and maintaining viability over extended assay procedures.
- Validation experiments, such as Z'-Factor and Lucifer Yellow, demonstrate that each automated assay process is robust and capable of yielding data superior to that produced through manual processing.
- Pharmacology testing shows that the EL406, combined with the Precision liquid handler and Synergy microplate readers, combine to make a simple, easy-to-use, and robust solution for automated processing of cell-based assays.

¹Zhang J. *et al.*: A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. *Journal for Biomolecular Screening* 1999 4(2): 67-73.

²Conn, G. *et al.*: Amino acid and cDNA sequences of a vascular endothelial cell mitogen that is homologous to platelet-derived growth factor. *Proceedings of the National Academy of Sciences USA* 1990 87: 2628-2632.