

# A High Content Homogeneous Assay for P-Glycoprotein Inhibition using Microplate Cytometry

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## Abstract

P-glycoprotein (Pgp) is an ATP-dependent transmembrane protein, that actively transports molecules out of cells, including drugs such as digoxin and HIV protease inhibitors. Screening for inhibitors of Pgp activity at an early stage can thus highlight potential drug-drug interactions.

Laser-scanning fluorescence microplate cytometers, such as the Acumen<sup>e</sup>X3 (TTP LabTech Ltd, Melbourn, UK), offer 405nm, 488nm and 633nm laser excitation in a single bench-top instrument. This technology is heavily used in oncology research and can provide rapid high content analysis of samples in microplate format. Thus the technology can be applied to kinetic fluorescence assays normally analysed by bulk readers. This is not possible on microscope-based CCD imagers due to their limited field of view and low speed. An Acumen<sup>e</sup>X3 can process 300,000 wells of data in 24 hours in 1536 plates. Typical analysis time in 96 well format is 10 minutes per plate including data processing and reporting.

Here, we describe the development of a microplate screening assay for Pgp activity and present data for three standard inhibitors in HepG2 cells. Cellular fluorescence resulting from Pgp uptake was measured using an Acumen<sup>e</sup>X3 microplate cytometer.

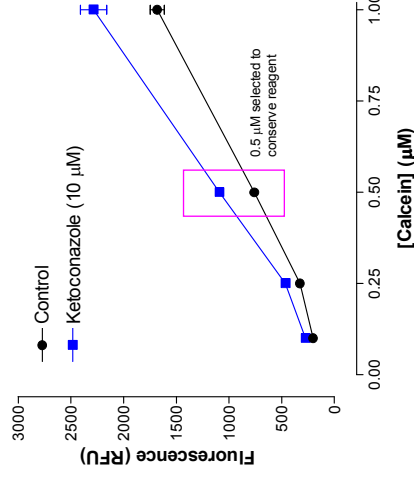
## Conclusion

- We have developed a microplate method to quantify Pgp activity using calcein AM and an Acumen<sup>e</sup>X3 microplate cytometer.
- The method successfully identified known inhibitors of Pgp as exemplified in HepG2 cells.
- Multiplexing of Pgp activity and cellular toxicity can provide valuable additional information on test compound activity and reduce false positives.
- The rapid data acquisition and whole well analysis capability of an Acumen<sup>e</sup>X3 make it an ideal technology for high throughput determination of Pgp activity.

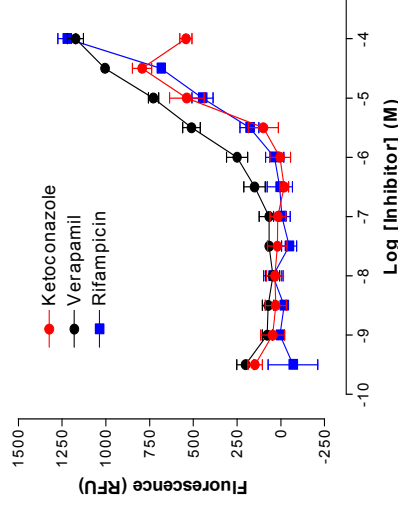
## 1 Assay Protocol

- Harvest HepG2 cells by Trypsin/EDTA and triturate to break up clumps.
- Seed at 2,000 cells per well in 96 (100  $\mu$ L) or 384 (50  $\mu$ L) well plates and incubate overnight @ 37°C / 5% CO<sub>2</sub>.
- Prepare 10 mM stocks of test compounds in DMSO.
- Prepare x10 stocks of test compounds in serum-free medium.
- Add test compounds and controls to give final drug concentrations of 1 nM to 100  $\mu$ M, and incubate for further 24 hours @ 37°C / 5% CO<sub>2</sub>. Upper limits of drug concentration may be limited by solubility in 1% DMSO.
- Add fluorescent dye solution to give final concentrations of 0.5  $\mu$ M Calcein AM, and 1.5  $\mu$ M propidium iodide (Molecular Probes).
- Incubate @ 37°C / 5% CO<sub>2</sub> for 2 hours.
- Load the plate into the Acumen<sup>e</sup>X3 and scan.

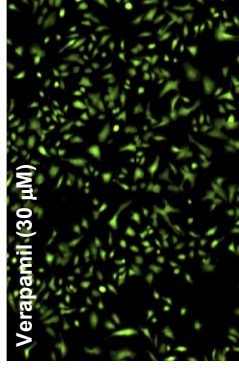
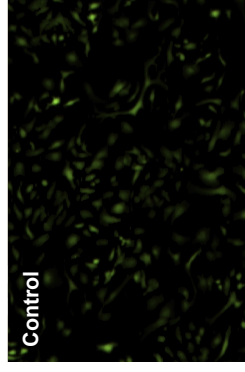
## 3 Optimisation of Calcein Concentration



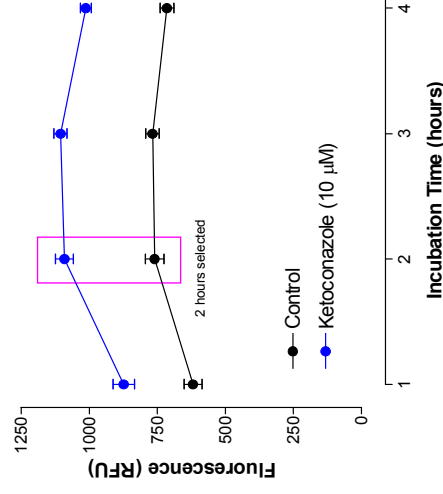
## 5 Effect of Pgp Inhibitors on Calcein Retention in 96 Well Plates



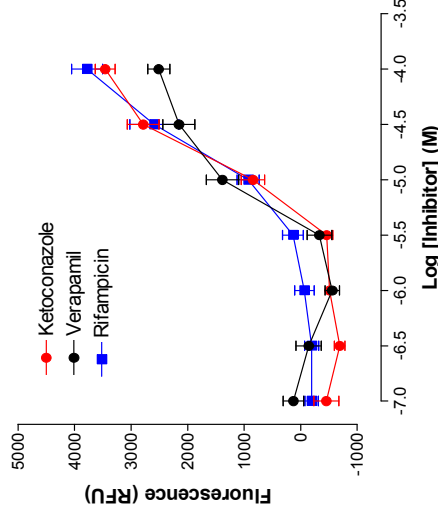
## 2 Well Views of Calcein Retention in Control and Verapamil-treated Wells



## 4 Optimisation of Incubation Time



## 6 Effect of Pgp Inhibitors on Calcein Retention in 384 Well Plates



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