

## **FLIPR®-based Assay for Screening Inhibitors of the Multidrug Resistance Gene MDR1 *In Vitro***

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The multidrug resistance (MDR1) gene product, P-glycoprotein (PGP), is responsible for the ATP-dependent extrusion of a variety of compounds, including chemotherapeutic agents, from cells (Abraham *et al.*, 1993). The role of inhibitors of the ATP-binding cassette (ABC) transporter-facilitated ATP transport and signalling in epithelial cell function is an area of research which could impact on disease pathology (Schwiebert, 1999) as well as the pharmacokinetics of new therapeutic agents. However, a high throughput *in vitro* assay for this target has been elusive. We report here a FLIPR-based assay for detection of inhibitors of human MDR1 based on a new reagent kit from Molecular Devices Corporation (MDC).

Cells with or without recombinant human MDR1, were maintained in Dulbecco's Modified Eagle's Media containing 10 % fetal calf serum (FCS) and 2 mM Glutamax I. Cells were seeded (30,000-50,000 cells per well) in 80µl media, with or without 10% FCS, into Costar 96 well black walled plates, and cultured overnight. The cells were then incubated at room temperature or 37°C for 20 min with Cyclosporin A (300 nM-100 µM), verapamil (300 nM-100 µM), probenecid (150 µM-30 mM) or buffer control. The cells were then placed in a FLIPR and the fluorescence monitored ( $\lambda_{EX}=488$  nm,  $\lambda_{EM}=540$  nm; Wood *et al.*, 2000) before and after the addition of the MDC reagent. Data reported are mean  $\pm$  s.e.mean where n= 3-9.

Following the addition of the MDC reagent there was an initial transient drop (an addition artefact), followed by a subsequent increase, in fluorescence. This increase in fluorescence was linear for at least 15 min and occurred in both wild type and MDR1-expressing cells, although the rate of increase was greater in the wild type cells ( $10.1\pm 0.3$  vs  $0.4\pm 0.2$  FIU s<sup>-1</sup>), confirming the MDC reagent was a MDR1 substrate. The responses were most robust in cells seeded at 50,000 cells per well in the presence of 10% FCS, and therefore these conditions were used for all compound studies. In MDR1-expressing cells the classic MDR1 inhibitors, verapamil and cyclosporin A increased the fluorescence in a concentration-dependent manner (pEC<sub>50</sub> values of  $5.35\pm 0.03$  &  $5.68\pm 0.01$  respectively), whilst probenecid, which inhibits other common extrusion pumps but not MDR1 (Wood *et al.*, 2000) and buffer control (1% DMSO) were without effect. Furthermore, the effects of verapamil and cyclosporin

A were temperature-dependent (pEC50 values at room temperature  $4.30 \pm 0.14$  &  $5.46 \pm 0.01$  and at  $37^{\circ}\text{C}$   $4.72 \pm 0.17$  &  $5.56 \pm 0.03$  respectively) and none of the compounds had an effect in wild type cells. This clearly indicates that the assay was specific to MDR1. Comparison of the potencies of cyclosporin A over several assays yielded intra- and inter-assay coefficients of 5.73% and 5.82% respectively, demonstrating the robustness of the assay

In conclusion, these data indicate that the MDC reagent allows the generation of a robust FLIPR-based assay for screening inhibitors of MDR1 *in vitro*.

Abraham, E.H. *et al.*, (1993) Proc. Natl. Acad.Sci. USA **90**, 312-316.

Schwiebert, E.M., (1999) Am. J. Physiology. **276**, C1-8.

Wood, M.D. *et al.*, (2000) Recent Res. Devel. Neurochem. **3**, 135-142.