

# Cloe Select P-glycoprotein Inhibition

## Background Information



'Bi-directional transport methodology is the preferred functional assay used to identify drugs as substrates and / or inhibitors of P-gp. These experiments require the use of known P-gp substrates and inhibitors.'

FDA Draft Guidance for Industry: Drug Interaction Studies - Study Design, Data Analysis, and Implications for Dosing and Labeling (September 2006)

- P-glycoprotein is one of the most well-recognised efflux transporters in many tissues including the intestine, brain and kidney.
- Inhibition of P-gp has shown to be responsible for several clinical drug-drug interactions. For example, clarithromycin can inhibit the transport of the P-gp substrate digoxin resulting in an elevation of plasma levels and a decrease in renal clearance<sup>1</sup>.
- MDR1-MDCK cells originate from transfection of Madin Darby canine kidney (MDCK) cells with the *MDR1* gene, the gene encoding for the efflux protein, P-glycoprotein (P-gp)<sup>2</sup>.
- The MDR1-MDCK cell line is a recommended *in vitro* model for determining the extent of P-gp inhibition of a compound.
- Cloe Select P-gp Inhibition assay meets criteria set in the FDA guidelines.

## Protocol

### Substrate

5  $\mu$ M Loperamide  
(FDA recommended substrate)

### Test Compound Concentrations

Single point or 8 point IC<sub>50</sub>

### Direction

Apical to Basolateral and Basolateral to Apical

### Incubation Time

60 min

### Growth Period

4 days

### Compound Requirements

100  $\mu$ L of 10 mM solution

### Analysis Method

LC-MS/MS quantification

### Integrity Marker

Lucifer Yellow

### Data Delivery

P<sub>app</sub> and efflux ratios in presence and absence of inhibitor (single point)  
IC<sub>50</sub>

Interference at the level of ATP binding cassette (ABC) and other transporters is increasingly being identified as the mechanism behind clinically important drug– drug interactions<sup>3</sup>.

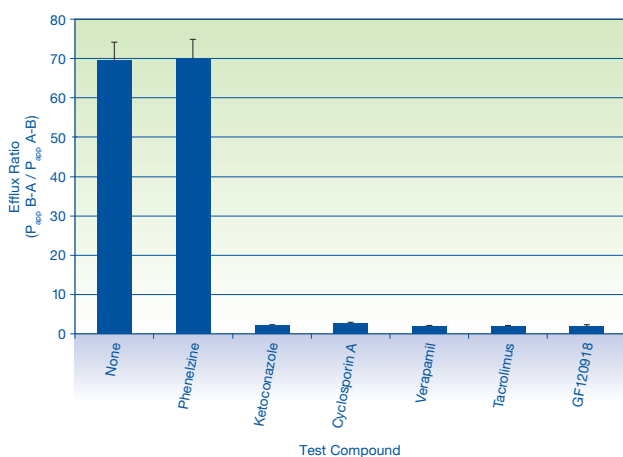


### Cloe Select P-gp Inhibition

A set of known P-gp inhibitors were investigated in the Cloe Select P-gp Inhibition assay using loperamide as substrate.

**Figure 1**

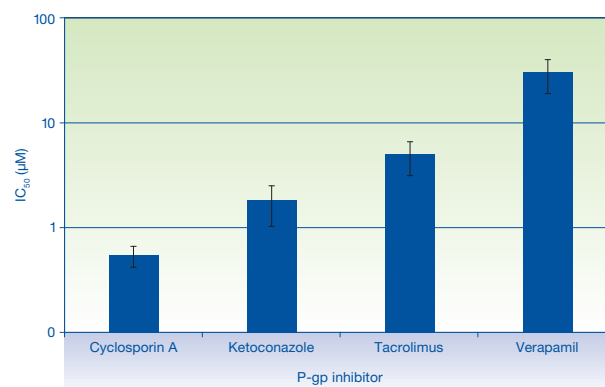
Cloe Select P-gp Inhibition data from single point assay illustrating efflux ratio of loperamide with and without known P-gp inhibitors.



Graph shows the inhibitory effect of known P-gp inhibitors (10  $\mu$ M cyclosporin A; 50  $\mu$ M ketoconazole; verapamil; tacrolimus; GF120918) and a negative control (50  $\mu$ M phenelzine) on the efflux of 5  $\mu$ M loperamide. Error bars represent the standard deviation from 3 replicates.

**Figure 2**

Cloe Select P-gp Inhibition data from the IC<sub>50</sub> assay determined by measuring the efflux ratio of loperamide in the presence of known P-gp inhibitors.



Graph shows the inhibitory effect of known P-gp inhibitors on the efflux ratio of 5  $\mu$ M loperamide. Error bars represent the standard deviation of 9 replicates.

#### References

- Wakasugi H *et al.* (1998) *Clin Pharmacol Ther* **64**; 123 -128.
- Pastan I *et al.* (1988) *Proc Natl Acad Sci USA* **85**; 4486-4490.
- Marchetti S *et al.* (2007) *Oncologist* **12**; 927-941.