

# Cloe Screen MDR1 - MDCK Permeability (P-glycoprotein Substrate Identification)

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## Background Information



'Cells used for bi-directional transport studies should form a functionally polarized cell monolayer, complete with tight junctions. At present, the preferred cells lines include Caco-2, transfected LLC-PK1-MDR1, and transfected MDCK-MDR1. LLC-PK1 and MDCK wild type cells are used as negative controls.'

FDA Draft Guidance for Industry - Drug Interaction Studies - Study Design, Data Analysis, and Implications for Dosing and Labeling (September 2006).

- 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>1</sup>.
- The MDR1-MDCK permeability assay is a valuable tool for the identification and characterisation of P-gp substrates and inhibitors.
- Using MDR1-MDCK cells avoids the complexities of multiple transporters by focusing specifically on P-gp, one of the most well recognized efflux transporters in many tissues including the brain, kidney and intestine.
- MDR1-MDCK helps to gain a greater understanding of the mechanism of drug efflux, and highlights early potential issues with drug permeability.
- MDR1-MDCK has been found to be a useful predictor of blood brain barrier permeability.

### Protocol

**Test Compound Concentration**  
10  $\mu$ M (different concentrations available)

**Direction**  
Apical to Basolateral and/or  
Basolateral to Apical

**Number of Replicates**  
2

**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**  
 $P_{app}$   
Efflux Ratio for Bidirectional Assessment

By assessing the transport in both the apical to basolateral and basolateral to apical direction an efflux ratio can be calculated which indicates if the compound is a substrate of P-gp.



### Cloe Screen MDR1-MDCK Permeability (P-gp Substrate Identification)

The Cloe Screen MDR1-MDCK Permeability assay is able to identify compounds which are substrates of P-gp and distinguish between compounds which are CNS negative and CNS positive.

**Table 1**

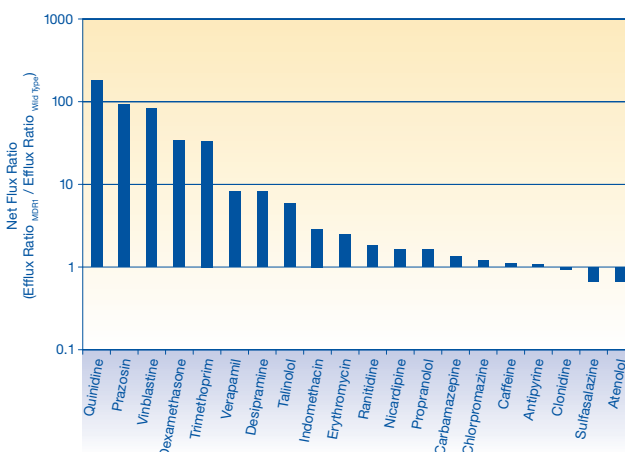
Classification of brain uptake using Cloe Screen MDR1-MDCK Permeability.

Drug	$P_{app, A-B}$ ( $\times 10^{-6}$ cm/s)	Brain Uptake Classification
Atenolol	0.204	CNS Negative <sup>2</sup>
Methotrexate	0.234	CNS Negative <sup>2</sup>
Ranitidine	0.369	CNS Negative <sup>2</sup>
Vinblastine	0.521	CNS Negative <sup>2</sup>
Cimetidine	0.522	CNS Negative <sup>3</sup>
Sulfasalazine	0.535	CNS Negative <sup>2</sup>
Quinidine	1.49	CNS Negative <sup>2</sup>
Loperamide	1.82	CNS Negative <sup>4</sup>
Minoxidil	2.77	CNS Negative <sup>5</sup>
Flecainide	3.50	CNS Positive <sup>6</sup>
Fluconazole	9.50	CNS Positive <sup>7</sup>
Acetaminophen	17.4	CNS Positive <sup>8</sup>
Desipramine	31.1	CNS Positive <sup>2</sup>
Indomethacin	35.6	CNS Positive <sup>2</sup>
Warfarin	40.7	CNS Positive <sup>9</sup>
Chlorpromazine	53.4	CNS Positive <sup>2</sup>
Propranolol	63.9	CNS Positive <sup>10</sup>
Carbamazepine	64.5	CNS Positive <sup>2</sup>
Antipyrine	67.7	CNS Positive <sup>2</sup>

Cloe Screen MDR1-MDCK distinguishes between CNS positive and CNS negative compounds based on their  $P_{app}$  values.

**Figure 2**

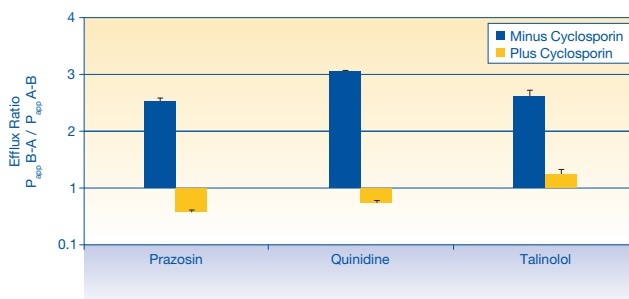
Net flux ratio for a set of 20 compounds (calculated using the efflux ratios of the wild type and MDR1-MDCK bidirectional assays).



By performing a bidirectional study in both the wild type and MDR1-MDCK assay, the net flux ratio can be calculated to identify compounds which are substrates of human P-glycoprotein.

**Figure 3**

Graph shows the effect of cyclosporin A (10  $\mu$ M) on the efflux of prazosin, quinidine and talinolol.



Cyclosporin A is an inhibitor of P-glycoprotein and inhibits the efflux of prazosin, quinidine and talinolol in the Cloe Screen MDR1-MDCK bidirectional assay.

#### References

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