

## In vitro ADME & PK

# MDCK-MDR1 Permeability Assay

## Background Information



'P-gp and BCRP are not expected to impact the oral bioavailability of highly permeable and highly soluble drugs'

<sup>1</sup>Draft FDA Guidance for Industry - In Vitro Metabolism- and Transporter-Mediated Drug-Drug Interaction Studies (October 2017)

- MDCK-MDR1 cells originate from transfection of Madin Darby canine kidney (MDCK) cells with the *MDR1* gene (ABCB1), the gene encoding for the efflux protein, P-glycoprotein (P-gp)<sup>2</sup>.
- Assessing transport in both directions (apical to basolateral (A-B) and basolateral to apical (B-A)) across the cell monolayer enables an efflux ratio to be determined which provides an indicator as to whether a compound undergoes active efflux (mediated by P-gp).
- MDCK-MDR1 helps to gain an understanding of the mechanism of drug efflux, and highlights early potential issues with drug permeability.
- In addition to intestinal permeability, MDCK-MDR1 permeability has also been found to be a useful predictor of blood brain barrier permeability.

### Protocol

**Test Article Concentration**

10  $\mu$ M

**Passage Number**

6 - 30

**Period of Cell Culture**

4 days

**Number of Replicates**

2

**Incubation Time**

60 min

**Temperature**

37°C

**Test Article Requirements**

100  $\mu$ L of 10 mM DMSO solution

**Integrity Marker**

Lucifer Yellow

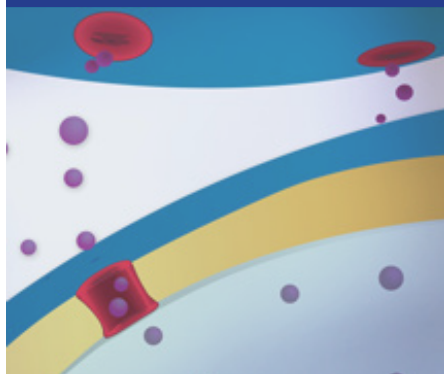
**Analysis Method**

LC-MS/MS quantification

**Data Delivery**

$P_{app}$   
Efflux Ratio  
% Recovery

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.



### MDCK-MDR1 Permeability

Cyprotex's MDCK-MDR1 permeability assay is able to identify compounds which are substrates of P-gp (See Figure 1) and distinguish between compounds which are CNS negative and CNS positive as shown in Table 1.

**Table 1**

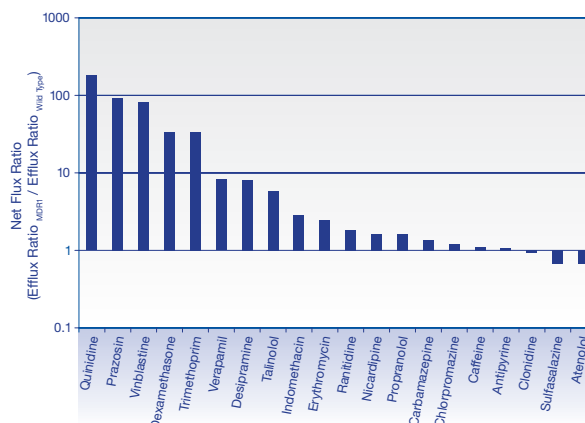
Classification of brain uptake using Cyprotex's MDCK-MDR1 permeability assay.

Drug	$P_{app} A-B$ ( $\times 10^{-6}$ cm/s)	Brain Uptake Classification
Atenolol	0.204	CNS Negative <sup>3</sup>
Methotrexate	0.234	CNS Negative <sup>3</sup>
Ranitidine	0.369	CNS Negative <sup>3</sup>
Vinblastine	0.521	CNS Negative <sup>3</sup>
Cimetidine	0.522	CNS Negative <sup>4</sup>
Sulfasalazine	0.535	CNS Negative <sup>3</sup>
Quinidine	1.49	CNS Negative <sup>3</sup>
Loperamide	1.82	CNS Negative <sup>5</sup>
Minoxidil	2.77	CNS Negative <sup>6</sup>
Flecainide	3.50	CNS Positive <sup>7</sup>
Fluconazole	9.50	CNS Positive <sup>8</sup>
Acetaminophen	17.4	CNS Positive <sup>9</sup>
Desipramine	31.1	CNS Positive <sup>3</sup>
Indomethacin	35.6	CNS Positive <sup>3</sup>
Warfarin	40.7	CNS Positive <sup>10</sup>
Chlorpromazine	53.4	CNS Positive <sup>3</sup>
Propranolol	63.9	CNS Positive <sup>11</sup>
Carbamazepine	64.5	CNS Positive <sup>3</sup>
Antipyrine	67.7	CNS Positive <sup>3</sup>

Cyprotex's MDCK-MDR1 assay distinguishes between CNS positive and CNS negative compounds based on their  $P_{app}$  values.

**Figure 1**

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



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

### References

- FDA Draft Guidance for Industry - Drug Interaction Studies - Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations (February 2012)
- Pastan I *et al.*, (1988) *Proc Natl Acad Sci USA* **85**; 4486-4490
- Wang O *et al.*, (2005) *Int J Pharmaceut* **288**; 349-359
- Di L *et al.* (2003) *Eur J Med Chem* **38**; 223-232
- Seeling A *et al.*, (1994) *Proc Natl Acad Sci USA* **91**; 68-72
- Thomas RC *et al.*, (1975) *J Pharm Sci* **64**; 1360-6
- Piovan D *et al.*, (1986) *Pharmacol Res Commun* **18**; 739-745
- Yang H *et al.*, (1996) *Pharm Res* **13**; 1570-5
- Courad JP *et al.*, (2001) *Life Sci* **69**; 1455-64
- Murakami H *et al.*, (2000) *Am J Physiol Heart Circ Physiol* **279**; H1022-1028
- Liu X *et al.*, (2004) *Drug Metab Dispos* **32**; 132-139