cyprotex AN EVOTEC COMPANY

In vitro **ADME & PK**

MDCK-MDR1 Permeability Assay

Background Information

'Bidirectional assays evaluate whether an investigational drug is a substrate or inhibitor of efflux transporters such as P-gp or BCRP.'

[1]FDA Guidance for Industry - In Vitro Metabolism- and Transporter-Mediated Drug-Drug Interaction Studies (January 2020)

- 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)[2].
- 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 µM

Passage Number
6 - 30

Period of Cell Culture
4 days

Number of Replicates
2

Incubation Time
60 min

Temperature
37°C

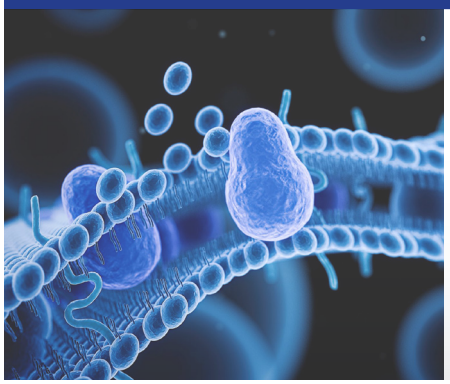
Test Article Requirements
100 µL of 10 mM DMSO solution

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 Permability

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

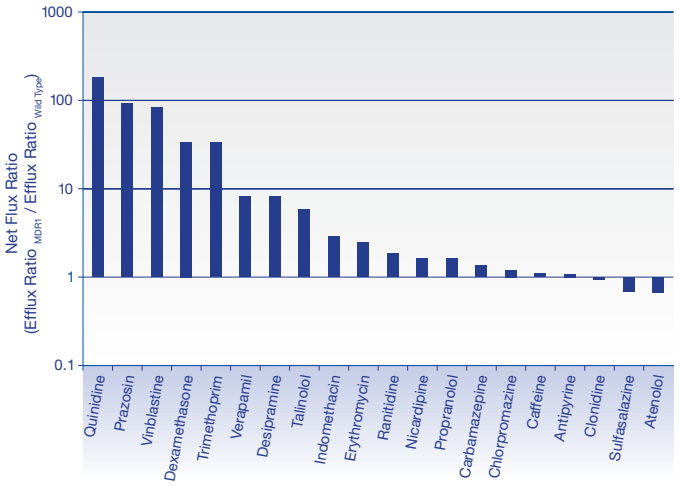
Classification of brain uptake using Cyprotex's MDCK-MDR1 permeability assay.

Drug	P_{app} A-B ($x10^{-6}$ cm/s)	Brain Uptake Classification
Atenolol	0.204	CNS Negative[3]
Methotrexate	0.234	CNS Negative[3]
Ranitidine	0.369	CNS Negative[3]
Vinblastine	0.521	CNS Negative[3]
Cimetidine	0.522	CNS Negative[4]
Sulfasalazine	0.535	CNS Negative[3]
Quinidine	1.49	CNS Negative[3]
Loperamide	1.82	CNS Negative[5]
Minoxidil	2.77	CNS Negative[6]
Flecainide	3.50	CNS Positive[7]
Fluconazole	9.50	CNS Positive[8]
Acetaminophen	17.4	CNS Positive[9]
Desipramine	31.1	CNS Positive[3]
Indomethacin	35.6	CNS Positive[3]
Warfarin	40.7	CNS Positive[10]
Chlorpromazine	53.4	CNS Positive[3]
Propranolol	63.9	CNS Positive[11]
Carbamazepine	64.5	CNS Positive[3]
Antipyrine	67.7	CNS Positive[3]

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

[1] FDA Guidance for Industry - In Vitro Drug Interaction Studies - Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions (January 2020)
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[3] Wang O *et al*, (2005) *Int J Pharmaceut* **288**; 349-359
[4] Di L *et al* (2003) *Eur J Med Chem* **38**; 223-232
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