Background Information

- 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).

- 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

To find out more contact enquiries@cyprotex.com
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.

<table>
<thead>
<tr>
<th>Drug</th>
<th>P&lt;sub&gt;app&lt;/sub&gt; A-B [x10&lt;sup&gt;-6&lt;/sup&gt; cm/s]</th>
<th>Brain Uptake Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atenolol</td>
<td>0.204</td>
<td>CNS Negative&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>0.234</td>
<td>CNS Negative&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>0.369</td>
<td>CNS Negative&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vinblastine</td>
<td>0.521</td>
<td>CNS Negative&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>0.522</td>
<td>CNS Negative&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sulfasalazine</td>
<td>0.535</td>
<td>CNS Negative&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Quinidine</td>
<td>1.49</td>
<td>CNS Negative&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Loperamide</td>
<td>1.82</td>
<td>CNS Negative&lt;sup&gt;6&lt;/sup&gt;</td>
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<td>Minoxidil</td>
<td>2.77</td>
<td>CNS Negative&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>Flecaïnide</td>
<td>3.50</td>
<td>CNS Positive&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>9.50</td>
<td>CNS Positive&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>17.4</td>
<td>CNS Positive&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Desipramine</td>
<td>31.1</td>
<td>CNS Positive&lt;sup&gt;4&lt;/sup&gt;</td>
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<td>Indomethacin</td>
<td>35.6</td>
<td>CNS Positive&lt;sup&gt;6&lt;/sup&gt;</td>
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<td>Warfarin</td>
<td>40.7</td>
<td>CNS Positive&lt;sup&gt;10&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>53.4</td>
<td>CNS Positive&lt;sup&gt;4&lt;/sup&gt;</td>
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<td>Propranolol</td>
<td>63.9</td>
<td>CNS Positive&lt;sup&gt;11&lt;/sup&gt;</td>
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<td>Carbamazepine</td>
<td>64.5</td>
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<td>Antipyrine</td>
<td>67.7</td>
<td>CNS Positive&lt;sup&gt;6&lt;/sup&gt;</td>
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</tbody>
</table>

Cyprotex's MDCK-MDR1 assay distinguishes between CNS positive and CNS negative compounds based on their P<sub>app</sub> 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 Draft Guidance for Industry - Drug Interaction Studies - Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations (February 2012)