Human P-Glycoprotein / MDR1
Drug Interaction Assay

P-Glycoprotein (P-gp)
Multi-Drug Resistance 1 (MDR1) Transporter
ATP Binding Cassette Protein B1 (ABCB1)

2x 48-well Assay Format
Product # HPGP-48

Technical Manual
(v2.0)

www.indigobiosciences.com
3006 Research Drive, Suite A1, State College, PA 16801, USA
Customer Service:
814-234-1919; FAX 814-272-0152
customerserv@indigobiosciences.com

Technical Service:
814-234-1919
techserv@indigobiosciences.com
Human P-Glycoprotein / MDR1 Drug Interaction Assay
2x 48-well Assay Format

I. Background .................................................................................................................. 3

II. Assay Description .................................................................................................... 3

III. Kit Components & Storage Conditions .................................................................... 5

IV. Materials to be Supplied by the User ..................................................................... 6

V. Assay Setup & Workflow .......................................................................................... 6
   ▪ Filter sets for measuring Daunorubicin Fluorescence .......................................... 6
   ▪ Assay Performance .................................................................................................. 8
   ▪ Preparation of Reference and Test Drugs .............................................................. 9
   ▪ Data Analyses ........................................................................................................... 9
   ▪ Assay Performance ................................................................................................. 5

VI. P-glycoprotein Drug Interaction Assay Protocol
   ▪ DAY 1 Assay Protocol ............................................................................................ 10
   ▪ DAY 2 Assay Protocol ............................................................................................ 11

VII. Limited Use Disclosures ......................................................................................... 12

APPENDIX 1: Example Scheme for Serial Dilutions ...................................................... 13

APPENDIX 2: Sample Calculations ............................................................................... 14
I. Background

P-glycoprotein (P-gp, aka MDR-1, or ABCB1) is a 170kDa transmembrane glycoprotein that functions as an ATP-dependent efflux transporter. P-gp is comprised of twelve membrane-spanning domains and two cytosolic nucleotide-binding domains. The transmembrane domains comprise several substrate binding pockets capable of interacting with a broad range of both endogenous and foreign small molecule chemotypes. Xenobiotic substrates of P-gp range from pollutants, such as those encountered through unintended exposure to industrial and agricultural chemicals, to small molecule drugs that are intentionally administered for therapeutic benefit.

P-gp is highly expressed in gastrointestinal epithelium, liver, pancreatic and kidney cells, as well as the capillary endothelial cells that establish the blood-brain barrier. In combination with the activities of Cytochrome P450 oxidases, the robust efflux activity of P-gp plays a critical role in limiting the absorption and systemic physiological distribution of xenobiotics, as well as facilitating their ultimate elimination from the body.

Determining if a drug candidate has incidental activity as either a substrate or an inhibitor of the P-gp transporter is an important component of the drug safety assessment process. A drug that is a P-gp substrate or, in particular, an inhibitor can profoundly alter the rate of absorption, distribution, metabolic conversion, and eventual excretion (ADME) of co-administered drugs, thereby significantly shifting their respective therapeutic efficacies and toxicologic profiles. Not surprisingly, assessing a drug’s potency as an interactor with P-gp, and thus its potential liability for inducing downstream drug-drug interactions (DDI), is mandated by the FDA.

II. Assay Description

This assay utilizes HCT-Pgp cells, a proprietary cell line derived from human colorectal adenocarcinoma cells that have been extensively selected for high-level expression of native P-glycoprotein. The primary application of this assay is to rapidly assess drug candidates as either inhibitors, substrates, or non-substrates of P-glycoprotein. This is accomplished by co-treating the cells with varying concentrations of the test drug and a fixed concentration of daunorubicin, a well characterized fluorescent substrate for P-glycoprotein mediated transport. A short incubation period allows cellular uptake of both the test drug and the fluorescent probe substrate, daunorubicin. The interaction of these two molecules with P-gp results in the active export of daunorubicin, and potentially the test drug, back to the extracellular environment. If the test drug is also a substrate for P-gp it will exert competition with daunorubicin for P-gp binding and export. At the assay endpoint the treatment media are removed, the cells are washed to remove extracellular daunorubicin, the cells are then lysed and fluorescence intensity is measured. Fluorescence derives from the number of daunorubicin molecules that have accumulated within the cells over the treatment period. Comparing the RFU (and EC50) values of ‘drug treated’ cells to ‘vehicle treated’ cells provides a relative measure of the impact of the test drug on P-gp mediated transport of the probe substrate daunorubicin.

The potential interplay between P-gp, daunorubicin and a test drug is depicted in Figure 1. Co-administered drugs that are non-substrates for P-gp will not impede the rapid export of daunorubicin back into the extracellular space. Consequently, the intracellular accumulation of daunorubicin will be very low, nearing background levels. Conversely, a test drug that is a substrate of P-gp will compete with daunorubicin for occupancy of the transporter’s binding pocket, resulting in reduced efficiency of efflux, and increased intracellular accumulation of daunorubicin. The relative potencies of test drugs as competitive substrates for P-gp may be ranked by comparing their respective EC50 values.

Finally, a co-administered drug that is an inhibitor of P-gp will stop transporter function, resulting in maximal intracellular accumulation of daunorubicin.

Comparing the dose-response metrics of vehicle-treated, drug-treated, and inhibitor (Tariquidar) treated HCT-Pgp cells allows one to determine if a drug is a non-substrate, a substrate, or an inhibitor of the P-gp transporter.
Figure 1. A.) Depiction of the potential competition between co-administered test drugs and daunorubicin for binding interactions with the P-gp efflux transporter. B.) Corresponding data trends. Measured RFU values derive from daunorubicin that has accumulated within the cells. Consequently, there is an inverse-relationship between the rate of probe efflux and measured intracellular fluorescence. Tariquidar is a potent reference inhibitor of P-glycoprotein.

A.

<table>
<thead>
<tr>
<th></th>
<th>Tariquidar</th>
<th>Drug 1</th>
<th>Drug 2</th>
<th>Drug 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>RFU (Vehicle-sub.)</td>
<td>0</td>
<td>2,000</td>
<td>4,000</td>
<td>6,000</td>
</tr>
<tr>
<td></td>
<td>8,000</td>
<td>10,000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B. Fluorescence Intensity from Accumulated Intracellular Daunorubicin
III. Kit Components & Storage Conditions

This Human P-glycoprotein Drug Interaction Assay is formatted to allow for two independent 48-well assay setups. The kit contains two aliquots of HCT-Pgp cells and two black 96-well assay plates. Each aliquot of cells provides sufficient volume to dispense 48 assay wells of HCT-Pgp cells into one 96-well plate. If desired, the two aliquots of HCT-Pgp cells may be thawed, combined to generate a single cell suspension, then dispensed into all wells of an assay plate.

In addition to HCT-Pgp cells and assay plates, this kit includes Cell Recovery Medium (CRM-p) used to perform a rapid thaw of the -80°C HCT-Pgp cells, Compound Screening Medium supplemented with daunorubicin (CSM+DR) used to prepare drug treatment media, Wash Buffer, two aliquots of Lysis Reagent, and the potent reference inhibitor Tariquidar.

Assay kits are shipped on dry ice. Upon receipt, individual kit components may be stored at the temperatures indicated on their respective labels. More convenient, however, is to keep all kit components together and simply store the entire kit at -80°C.

To ensure maximal performance care should be taken to ensure that the HCT-Pgp cells are continuously maintained at no warmer than -76°C until immediately prior to use. Transient warming will degrade cell viability and assay performance. Even during transit to the laminar-flow hood the tube(s) of HCT-Pgp cells should go from the -80°C freezer directly into dry ice.

Each aliquot of HCT-Pgp Cells is provided as a single-use assay reagent. These cells have limited proliferative capacity; once thawed they cannot be maintained in extended culture. The date of product expiration is printed on the Product Qualification Insert (PQI) enclosed with each kit.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>• HCT-Pgp Cells</td>
<td>2 x 1.0 mL</td>
<td>-80°C</td>
<td>dry ice</td>
</tr>
<tr>
<td>• Cell Recovery Medium (CRM-p)</td>
<td>1 x 25 mL</td>
<td>-20°C</td>
<td>37°C</td>
</tr>
<tr>
<td>• Compound Screening Medium + Daunorubicin (CSM+DR)</td>
<td>1 x 50 mL</td>
<td>-20°C</td>
<td>r.t.</td>
</tr>
<tr>
<td>• Wash Buffer</td>
<td>1 x 55 mL</td>
<td>-20°C</td>
<td>on ice</td>
</tr>
<tr>
<td>• Lysis Reagent</td>
<td>2 x 7 mL</td>
<td>-20°C</td>
<td>r.t.</td>
</tr>
<tr>
<td>• Tariquidar, 100 µM (in DMSO) (inhibitor of P-gp; 100x conc.)</td>
<td>1 x 30 µL</td>
<td>-20°C</td>
<td>r.t.</td>
</tr>
<tr>
<td>• 96-well Assay Plates (Black, sterile, collagen-coated)</td>
<td>2</td>
<td>-20°C</td>
<td>r.t.</td>
</tr>
</tbody>
</table>

NOTE: This assay kit contains two 96-well assay plate in which the assay wells have been collagen-coated and dried; assay plates should be stored frozen (-20°C or colder) until use.
**IV. Materials to be Supplied by the User**

The following materials must be provided by the user, and should be made ready prior to initiating the assay procedure:

**Day 1**
- dry ice bucket
- 37°C water bath
- cell culture-rated laminar flow hood.
- 37°C, humidified 5% CO₂ incubator for mammalian cell culture.
- 70% alcohol wipes
- 8-channel pipettes suitable for dispensing 200 µl volumes; sterile tips
- disposable media reservoirs, sterile

**Day 2**
- DMSO
- wet ice bucket
- 8-channel pipettes (preferably electronic, repeat-dispensing) suitable for dispensing 100 µl volumes; sterile tips
- disposable media reservoirs, sterile.
- sterile multi-chambered media basins (such as the Heathrow Scientific "Dual-Function Solution Basin"), or deep-well plates, or appropriate similar vessel for generating dilution series of reference and test compound(s).
- Plate-reading Fluorometer (see “A word about filter sets for measuring Daunorubicin fluorescence”, below.

**V. Assay Setup & Workflow**

*Figure 2* provides an overview of the assay work flow. A detailed, step-by-step protocol for performing the P-gp / Drug Interaction Assay begins on page 10.

There are several possible configurations for assay plate setup, the most efficient of which will be dictated by the number of treatment concentrations and replicates desired by the user. For example, the plate setup depicted in *Figure 2* is practical when performing a preliminary screen of multiple compounds to assess if they do, or do not, interact with P-gp. Alternatively, full dose-response analyses, as depicted in *Figure 3*, provide a more complete assessment of a drug’s status as an interactor with P-gp. Importantly, determining EC₅₀ values allows one to discriminate between inhibitors and substrates of P-gp, as well as to rank the relative potencies of drugs that are P-gp substrates.

The assay workflow, *in brief*: A suspension of HCT-Pgp cells is prepared from one (or two) frozen tube(s) of cells and dispensed into 48 (or 96) wells of an assay plate. The plate is incubated for 24 – 26 hr to allow complete post-thaw recovery of the cells. Culture media are discarded and the prepared test drug(s), tariquidar, and vehicle treatment media are dispensed into appropriate assay wells and the assay plate is placed in a cell culture incubator for 2 hr. The P-gp/drug interaction reaction is terminated by discarding the treatment media, washing the cells with ice cold Wash Buffer, then adding Lysis Reagent to the assay wells. After a minimum 30 minute rest period, RFU values are quantified from all assay wells using a plate reading fluorometer.

- **Filter sets for measuring Daunorubicin Fluorescence**

  Maximal excitation of daunorubicin occurs at approximately 490 nm. Corresponding emission occurs over an extended range of wavelengths, featuring two overlapping peaks at approximately 560 and 590 nM. This allows flexibility in the selection of emission filters. INDIGO uses, and recommends, the following combination of filters:

  \[
  \text{Ex} \ 495/10\text{nm} : \text{Em} \ 595/35\text{nm}
  \]

  This filter combination provides a large separation between excitation and emission wavelengths, thereby reducing the probability of signal interference from drugs that have inherent fluorescence properties.

  The following alternative filter combinations have also been validated for quantifying daunorubicin fluorescence:

  \[
  \begin{align*}
  \text{Ex} & \ 495/10\text{nm} : \text{Em} \ 550/10\text{nm} \\
  \text{Ex} & \ 495/10\text{nm} : \text{Em} \ 580/25\text{nm}
  \end{align*}
  \]
Figure 2. Assay workflow. The preferred configuration of plate setup will depend on the experimental objective. The configuration depicted here is practical for a preliminary assessment of several test drugs to determine their potential interactions with the P-gp transporter. Working concentrations of Test Drugs and Controls are prepared in ‘CSM+DR’, a media formulation that includes the fluorescent substrate, daunorubicin (DR).
**Figure 3.** The effect of drug treatments on the intracellular accumulation of daunorubicin in HCT-Pgp cells. Increasing concentrations of drugs that are substrates for P-gp compete with daunorubicin for transporter occupancy, resulting in corresponding increases in intracellular accumulation of the fluorescent probe. HCT-Pgp cells provide low-nanomolar sensitivities to drugs that are inhibitors of P-gp. The reference inhibitor Tariquidar is provided with this assay kit.

- Assay Performance -

![Human P-Glycoprotein Drug Interaction Assay Measuring Intracellular Accumulation of Daunorubicin]

<table>
<thead>
<tr>
<th>Drug</th>
<th>EC50, µM</th>
<th>Drug Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tariquidar</td>
<td>0.023</td>
<td>Inhibitors of P-gp</td>
</tr>
<tr>
<td>LY335979 (Zosuquidar)</td>
<td>0.029</td>
<td>Inhibitor/Substrate</td>
</tr>
<tr>
<td>GF120918 (Elacridar)</td>
<td>0.079</td>
<td></td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>Verapamil</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Quinidine</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Digitoxin</td>
<td>81</td>
<td></td>
</tr>
</tbody>
</table>

Maximum P-gp mediated efflux of Daunorubicin
• Preparation of Reference and Test Drugs •

On Day 2 of the assay protocol, treatment media containing the various test drug(s) concentrations, the reference inhibitor Tariquidar, and the ‘vehicle only’ control media, should be prepared prior to removing the Day 1 assay plate from the cell culture incubator.

Test compounds are typically solvated at high-concentration in DMSO and stored frozen as master stocks. To reduce the occurrence of drug insolubility it is recommended that all master stocks are further diluted using DMSO (provided by the user) to generate 100x-concentrated stocks relative to all final concentrations of treatment media to be applied to the assay plate, as depicted in APPENDIX 1. For example, if the desired assay concentration of a drug is 400 µM, 20 µM and 1.0 µM, then use DMSO to prepare 40 mM, 2.0 mM and 0.1 mM intermediate solutions. The provided Compound Screening Medium supplemented with daunorubicin (CSM+DR) is then used as diluent to make the final 100-fold dilutions of each intermediate DMSO solution. At Step12 of the assay protocol 100 µl per well of each prepared treatment media is dispensed into the assay plate containing pre-cultured HCT-Pgp cells.

NOTE: the 100x intermediate stocks prepared in DMSO may be stored at -20°C for repeated use. However, test compounds may lack long-term solubility once diluted into the aqueous environment of CSM+DR. Therefore, all preparations of treatment media should be considered as 'single-use' reagents.

• Data Analyses •

Data reduction may be performed by different methods. However, we find that plotting ‘vehicle-subtracted RFU’ vs. ‘drug concentration’ is the most straight forward way to assess the potential interaction of a test drug with the P-gp transporter (for examples, see Figure 3, and Appendix 2).

Because “Vehicle-treated” HCT-Pgp cells deliver maximal P-gp efflux capacity, the majority of daunorubicin molecules will be excluded from intracellular accumulation. At the assay endpoint the extracellular daunorubicin is washed away. Measured RFU values originate from non-specific absorption of daunorubicin and fluorescence inherent to the plasticware. For these reasons, RFU values measured from vehicle-treated cells may be considered as ‘background’ signal. As demonstrated in Appendix 2, averaged RFU values calculated from the ‘vehicle’ control group should be subtracted from the averaged RFU values calculated from the respective treatment groups of all other test drugs.

As a reminder: Fluorescence intensity will have an inverse-correlation to the rate of P-gp mediated efflux of daunorubicin. Specifically, vehicle treated HCT-Pgp cells will exhibit maximal P-gp efflux capacity, resulting in the lowest intracellular accumulation of daunorubicin, and the lowest measured RFU values. A drug that is a substrate of P-gp will compete with daunorubicin for occupancy of the transporter’s binding pockets. This will result in reduced export of the probe, a corresponding increase in its intracellular accumulation and, consequently, an increase in measured RFU values. A drug that is an inhibitor of P-gp will block transporter activity, leading to maximal intracellular accumulation of daunorubicin and high RFU values.
**IV. P-glycoprotein Drug Interaction Assay Protocol**

Review the entire Assay Protocol before starting. The Day 1 workflow requires minimal time to prepare a cell suspension and plate the cells, which are then incubated for approximately 24 hr. The Day 2 workflow requires less than 2 hours to prepare test compound dilutions and process the Pgp assay plate(s).

**DAY 1:** All steps are to be performed using aseptic technique.

1.) Remove the **Cell Recovery Medium (CRM-p)** from freezer storage, thaw and equilibrate to $37^\circ C$ using a water bath. Also, equilibrate to room temperature the black 96-well plate.

2.) Retrieve **HCT-Pgp Cells** from -80°C storage and immerse in dry ice to transport the tube(s) to a laminar flow hood. Retrieve 1 tube if setting up 48 assay wells in one assay plate, or 2 tubes if intending to set up 96 assay wells using one or both assay plates.

3.) Retrieve **CRM-p** from the $37^\circ C$ water bath and sanitize the outside surface of the tube with a 70% ethanol swab.

4.) When ready to proceed, place the tube(s) of cells in a rack and, *without delay*, perform a rapid thaw of the frozen cells by transferring 11 ml of 37°C CRM-p directly into a single tube of frozen cells. Recap the tube of cells and immediately place it in a 37°C water bath for approximately 10 minutes. The resulting volume of cell suspension will be 12 ml per tube.

5.) Retrieve the tube(s) of HCT-Pgp cell suspension from the water bath and sanitize the outside surface with a 70% alcohol swab.

6.) *Gently* invert the tube(s) to gain homogenous cell suspensions, then transfer them into a sterile reservoir. Use an 8-channel pipette to dispense 200 µl / well of cell suspension into the assay plate.

   *NOTE:* Take care to prevent cells from settling in the reservoir during dispensing. Cell settling will result in increased well-to-well variation.

7.) Place the assay plate into a 37°C, $\geq 85\%$ humidity, 5% CO$_2$ incubator for 24 - 26 hours.

8.) For greater convenience on Day 2, retrieve **CSM+DR, Wash Buffer** and **Lysis Reagent** from freezer storage and place them in a dark refrigerator (+4°C) to thaw overnight.
9.) Retrieve the thawed CSM+DR and Lysis Reagent from the refrigerator and allow each to equilibrate to room temperature. Retrieve the thawed Wash Buffer from the refrigerator and immerse in wet ice.

10.) Use CSM+DR to prepare sufficient volumes* of the desired treatment media (refer to “Preparation of Reference and Test Compounds”, pg. 9). In general, prepare treatment media containing:

i.) the desired final treatment concentrations of test drug(s),

ii.) vehicle control treatment media, and

iii.) Tariquidar, a reference inhibitor of P-gp.

a.) 1 µM Tariquidar inhibits P-gp activity, providing a positive control for preliminary drug screens (such as depicted in Figure 2). Alternatively,

b.) if a full dose-response analyses of test compounds is desired, the individual dilution regimens depicted in Appendix I provide a dilution template for both Tariquidar and test drugs.

* In Step 12, 100 µl of the various treatment media preparations will be dispensed into replicate assay wells. Manage dilution volumes carefully; this assay kit provides 50 ml of CSM+DR.

Hold the prepared treatment media at room temperature, preferably in a low-light environment, until they are used in Step 15.

11.) At the end of the 24-26 hr cell recovery period, discard the culture media from the assay plate (no rinse required).

NOTE: Media discard may be quickly and efficiently performed by manually dumping-off the contents of the assay wells, then blotting away residual drops by lightly tamping the inverted plate onto a clean paper towel. The HCT-Pgp cells will remain tightly adhered to the bottom of the assay wells. At this point, aseptic technique is not required for handling the culture plate.

If the use of an aspiration device is preferred, take care to avoid touching the bottom of the wells or running the aspiration tip around the bottom circumference of the wells. Such practices will result in destruction of the cells and will greatly increase well-to-well variability. It is not necessary to remove trace amounts of liquid from the assay wells.

12.) Dispense 100 µl of the prepared treatment and control media into appropriate wells containing HCT-Pgp cells. Incubate the plate at 37°C for 2 hours.

13.) At the 2 hour timepoint discard the treatment media (as before in Step 11) and use ice cold Wash Buffer to perform a total of three rinses of the assay wells, as follows:

a.) dispense 100 µl / well Wash Buffer (0°C) → rock the plate back-and-forth one time → discard wash buffer, then …

b.) repeat with two additional rounds of ‘dispense, rock & discard’

14.) Dispense 100 µl / well of Lysis Reagent. Rock the plate back-and-forth one time, then allow the plate to rest at room temperature, under subdue light (e.g., covered, or in a drawer), for approximately 30’ minutes.

NOTE: Before the end of the lysis period, turn on the plate-reader and select the appropriate fluorescence-mode program. INDIGO recommends the filter combination:

Ex 495/10nm : Em 595/35nm

Note that alternative emission filters may be used; see ‘Filter sets for measuring Daunorubicin Fluorescence’, pg. 6.

15.) Quantify RFU values.

16.) Data analyses; refer to “Data Analyses”, pg. 9, and Appendix 2.
VI. Limited Use Disclosures

Products commercialized by INDIGO Biosciences, Inc. are for RESEARCH PURPOSES ONLY – not for therapeutic or diagnostic use in humans or animals.

Product prices, availability, specifications and claims are subject to change without prior notice. Please refer to INDIGO Biosciences website for updated product offerings.

Copyright © INDIGO Biosciences, Inc. (State College, PA, USA). All Rights Reserved.
APPENDIX 1. Example dilution scheme and plate setup for full dose-response assessment of drug interactions with P-gp, allowing for EC50 determinations.
Appendix 2. Sample Calculations and dose-response plots.

**Equation 1:**

\[
\text{Ave. RFU}_{\text{Drug}} - \text{Ave. RFU}_{\text{Vehicle}} 
\]

<table>
<thead>
<tr>
<th>Vehicle treated</th>
<th>Average RFU</th>
<th>Ave. RFU Vehicle-Subtracted</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% DMSO</td>
<td>4,934</td>
<td>0</td>
</tr>
</tbody>
</table>

**Tariquidar, mM**

<table>
<thead>
<tr>
<th>Dose (mM)</th>
<th>RFU</th>
<th>RFU Vehicle-Subtracted</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000262</td>
<td>5,076</td>
<td>142</td>
</tr>
<tr>
<td>0.000655</td>
<td>5,055</td>
<td>121</td>
</tr>
<tr>
<td>0.00164</td>
<td>5,089</td>
<td>155</td>
</tr>
<tr>
<td>0.00410</td>
<td>5,234</td>
<td>300</td>
</tr>
<tr>
<td>0.0102</td>
<td>7,906</td>
<td>2,972</td>
</tr>
<tr>
<td>0.0256</td>
<td>28,840</td>
<td>23,906</td>
</tr>
<tr>
<td>0.064</td>
<td>43,319</td>
<td>38,385</td>
</tr>
<tr>
<td>0.160</td>
<td>45,361</td>
<td>40,427</td>
</tr>
<tr>
<td>0.400</td>
<td>45,166</td>
<td>40,232</td>
</tr>
<tr>
<td>1.00</td>
<td>46,117</td>
<td>41,183</td>
</tr>
</tbody>
</table>

**Test Drug X, mM**

<table>
<thead>
<tr>
<th>Dose (mM)</th>
<th>RFU</th>
<th>RFU Vehicle-Subtracted</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0168</td>
<td>5,105</td>
<td>171</td>
</tr>
<tr>
<td>0.0419</td>
<td>5,133</td>
<td>199</td>
</tr>
<tr>
<td>0.105</td>
<td>5,273</td>
<td>339</td>
</tr>
<tr>
<td>0.262</td>
<td>5,453</td>
<td>519</td>
</tr>
<tr>
<td>0.655</td>
<td>5,841</td>
<td>907</td>
</tr>
<tr>
<td>1.64</td>
<td>8,744</td>
<td>3,810</td>
</tr>
<tr>
<td>4.10</td>
<td>17,658</td>
<td>12,724</td>
</tr>
<tr>
<td>10.2</td>
<td>31,615</td>
<td>26,681</td>
</tr>
<tr>
<td>25.6</td>
<td>40,768</td>
<td>35,834</td>
</tr>
<tr>
<td>64.0</td>
<td>43,651</td>
<td>38,717</td>
</tr>
<tr>
<td>160</td>
<td>44385</td>
<td>39,451</td>
</tr>
<tr>
<td>400</td>
<td>45034</td>
<td>40,100</td>
</tr>
</tbody>
</table>

**P-gp Drug Interaction Assay**

- **Tariquidar**
  - EC$_{50}$ = 0.023 µM
- **Drug X**
  - EC$_{50}$ = 6.6 µM