

## P-GP INTERACTION ASSESSMENT WITH CACO-2 OR MDR1-LLC-PK<sub>1</sub> CELL MONOLAYERS

### Permeability testing and P-gp transport and inhibition screening across Caco-2 and MDR1-LLC-PK<sub>1</sub> monolayers

Bidirectional transport assays across Caco-2 and MDR1-LLC-PK1 monolayers comply with the FDA recommended approach to determine apparent permeability of a test article and assess P-gp mediated transport and inhibition. Only BD Gentest<sup>SM</sup> Contract Research Services delivers a combination of industry leading proprietary products, advanced technology, expert guidance from renowned study directors and reliable, submission ready results. Together, these elements provide you with the most rapid path to more sound decision making in your drug discovery endeavors.

The **most rapid** path to more sound decision making.

#### CACO-2 IS THE IN VITRO GOLD STANDARD METHOD TO EVALUATE TEST ARTICLE PERMEABILITY (P<sub>app</sub>)

An important factor in oral bioavailability is the ability of a compound to be well absorbed in the small intestine. Polarized cell monolayers have become the gold standard in vitro test systems to quickly and cost effectively assess the permeability of a test article. Caco-2 cells resemble small intestinal epithelial cells in morphology and expression of certain enzymes and transporters. It is the most frequently used cell line for permeability testing and the recommended approach to rank order compounds according to the FDA's Biopharmaceutics Classification System (BCS) as low, medium, or high permeability compounds [1].

#### FDA RECOMMENDS BIDIRECTIONAL TRANSPORT ASSAYS IN POLARIZED CELL MODELS TO IDENTIFY SUBSTRATES AND INHIBITORS OF P-GLYCOPROTEIN

Transporter proteins expressed in various tissues including intestinal epithelium, kidney, liver, and blood-brain barrier are recognized for their effects on drug disposition. Current-FDA guidance<sup>[2]</sup> recommends the testing of investigational drugs for interactions with P-glycoprotein (P-gp) using bidirectional transport assays in polarized cell models. Caco-2 and MDR1-LLC-PK<sub>1</sub> cells both show high levels of P-gp activity making them excellent models for P-gp-mediated drug transport studies.

#### CACO-2 AND MDR1-LLC-PK1 CELL LINES ARE WELL CHARACTERIZED FOR EFFLUX TRANSPORTER ACTIVITY

Efflux transporters other than P-gp, such as breast cancer resistance protein (BCRP) and multidrug resistance-associated protein (MRP2) can also be expressed in commonly used cell monolayer models such as Caco-2 and LLC-PK<sub>1</sub> and contribute to observed efflux of a test article. BD's Caco-2 and MDR1-LLC-PK<sub>1</sub> cells are characterized for P-gp, BCRP, and MRP2 activity facilitating interpretation of efflux results.

[More on reverse](#)

#### KEY FEATURES

- Provides FDA recommended method for the determination of test article permeability and identification of P-gp substrates and inhibitors [1,2]
- Available using Caco-2 and LLC-PK<sub>1</sub> (P-gp expressing and control) cell monolayers
- Uses cell lines characterized for P-gp, BCRP, and MRP2 activity allowing reliable identification of efflux substrates and inhibitors
- Quantitation using LC/MS/MS or liquid scintillation counting
- Long-term reproducibility of permeability and efflux results for positive controls and comparators in both Caco-2 and LLC-PK<sub>1</sub> cell lines

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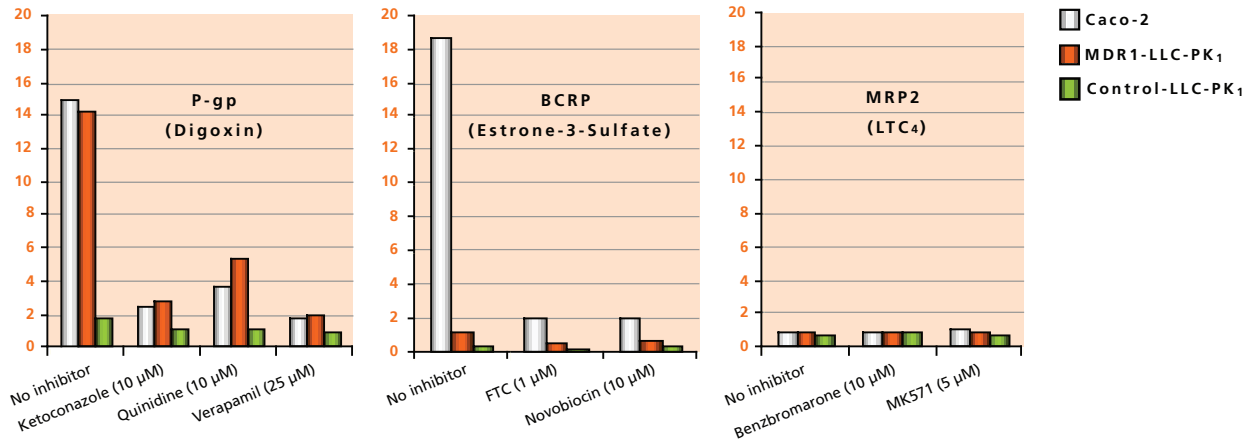
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## SPECIFICATIONS:

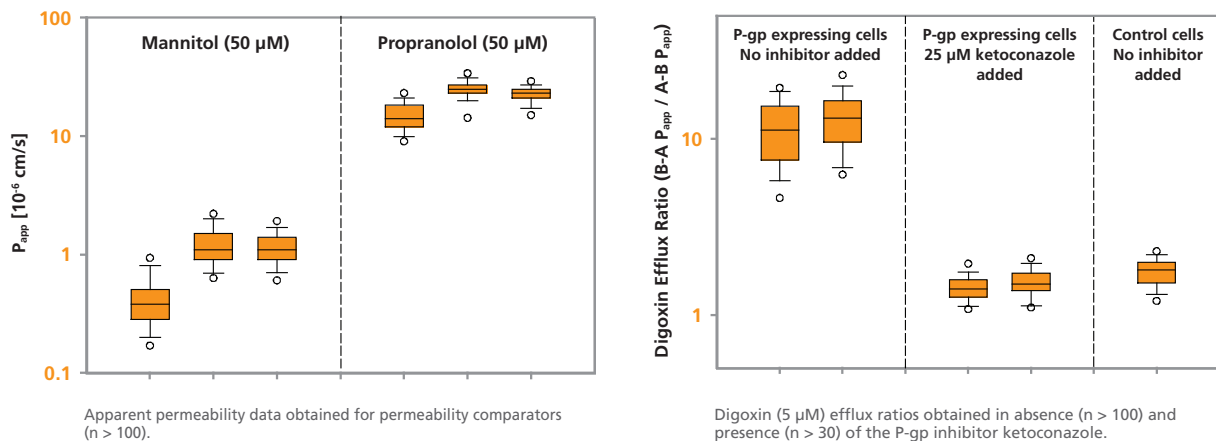
### EFFLUX TRANSPORTER ACTIVITY IN CACO-2 AND LLC-PK<sub>1</sub> CELL MONOLAYERS



**P-gp, BCRP, and MRP2 transporter activity assessment in Caco-2 and LLC-PK<sub>1</sub> cell monolayers.** Data represents efflux ratios of each probe substrate and the effects of prototypical inhibitors on the activity of each transporter. Efflux ratios were generated from mean A-B and B-A  $P_{app}$  values of duplicate monolayers.

Efflux of the P-gp probe substrate digoxin was observed in both Caco-2 and MDR1-LLC-PK<sub>1</sub> cells, and can be inhibited by the known P-gp inhibitors ketoconazole, quinidine, and verapamil. Efflux of the BCRP probe substrate estrone-3-sulfate (E3S) was observed in Caco-2 cells only, with an efflux ratio similar to that of digoxin. E3S efflux is significantly inhibited by the BCRP inhibitors novobiocin and fumitremorgin C (FTC). No efflux was observed for the MRP2 substrate LTC<sub>4</sub> in either Caco-2 or MDR1-LLC-PK<sub>1</sub> cells. LLC-PK<sub>1</sub> control cells showed no efflux activity for any of the probe substrates.

### HISTORICAL ASSAY PERFORMANCE FOR LOW AND HIGH PERMEABILITY COMPARATORS AND DIGOXIN EFFLUX AND INHIBITION IN CACO-2 AND LLC-PK<sub>1</sub> CELLS



Boxes represent the 25th-75th percentile, the line marks the median, error bars indicate the 90th and 10th percentiles, and circles are outliers outside the 5th/95th percentiles. Data were obtained using BD Falcon™ 24 multiwell inserts (1 μM PET membranes).

## STUDY DESIGN SUMMARY

TEST SYSTEM:	Caco-2 or LLC-PK <sub>1</sub> cell monolayers.
MONOLAYER QC:	TEER, lucifer yellow flux.
CONTROLS:	Low BCS <sup>[1]</sup> comparator: mannitol. High BCS <sup>[1]</sup> comparator: propranolol or metoprolol. P-gp substrate <sup>[2]</sup> : digoxin. P-gp inhibitors <sup>[2]</sup> : multiple available (e.g. ketoconazole, verapamil).
INCUBATION TIME:	90 min.
CONCENTRATIONS:	Single test article concentration, in duplicate.
ANALYTICAL METHOD:	LC/MS/MS.
REPORT:	A to B and B to A P <sub>app</sub> , efflux ratio, mass balance Excel data report.

### References

- [1] U.S. FDA/CDER, Biopharmaceutics Classification System (BCS), August 2000.
- [2] U.S. FDA/CDER, Drug Interaction Studies - Study Design, Data Analysis, and Implications for Dosing and Labeling, DRAFT GUIDANCE, September 2006.
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- [5] Stewart, B.H., et al., (1995) Comparison of intestinal permeabilities in multiple in vitro and in situ models: relationship to absorption in humans. *Pharm Res* 12:693.
- [6] Schwab, D., et al., (2003) Comparison of in Vitro P-Glycoprotein Screening Assays: Recommendations for Their Use in Drug Discovery *J. Med. Chem.*, 49:1716-1725.
- [7] Yamazaki, M., et al., (2001) In vitro substrate identification studies for P-glycoprotein-mediated transport: Species difference and predictability of in vivo results *J Pharmacol Exp Ther*, 296:723-735
- [8] Polli JW, et al. (2001). Rational use of in vitro P-glycoprotein assays in drug discovery. *J Pharmacol Exp Ther* 299:620.