

P-gp MEDIATED DRUG-DRUG INTERACTION

P-glycoprotein (P-gp), is an efflux pump located in the intestine and blood-brain barrier among other tissues. Compounds that are substrates for P-gp may be secreted back into the lumen of the intestine, resulting in poor absorption and bioavailability. Additionally, drugs that are targeted to the central nervous system, but are P-gp substrates, may be excluded from the brain, resulting in poor brain penetration.

To evaluate whether a compound is a P-gp substrate, it is important to calculate the efflux ratio (E ratio). The E ratio is the permeability value in the B-A direction divided by the permeability value in the A-B direction. Compounds with an E ratio greater than two are possible P-gp substrates. Further testing of A-B and B-A permeability in the presence of verapamil, a known P-gp inhibitor, can indicate whether the efflux is P-gp mediated.

Additionally, compounds may be inhibitors of P-gp, and interfere with the efflux of concomitantly administered drugs resulting in potentially toxic levels and severe side effects. Cerep offers two assays to evaluate possible P-gp inhibitors.

Compounds that are P-gp substrates may or may not inhibit P-gp activity. Similarly, compounds that are P-gp inhibitors may or may not be substrates for P-gp. At Cerep, assays are designed to specifically identify either P-gp substrates or P-gp inhibitors. The TC7 cell line, which contains endogenously high levels of P-gp activity, and the MDR1-MDCKII cell line, which over-expresses P-gp activity, are used as *in vitro* models to address P-gp mediated drug interactions.

DO YOUR COMPOUNDS INTERACT WITH P-GLYCOPROTEIN?

Compounds that are P-gp substrates are likely to be effluxed from the intestine resulting in poor intestinal permeability and limited absorption. These compounds may also be unable to penetrate the blood-brain barrier, which is critical for CNS targeted drugs.

Compounds that inhibit P-gp can result in a toxic build-up of concomitantly administered drugs that are P-gp substrates with potentially severe side effects.

EFFLUX RATIO TO EVALUATE P-gp SUBSTRATES

The E ratio describes the net flux across the monolayer. It is calculated as the ratio of B-A permeability to A-B permeability. A compound with an efflux ratio greater than 2 is typically considered a possible P-gp substrate.

The permeability assays are performed as follows. Test compounds are assayed in duplicate in 96 transwell plates. Compounds are prepared at 10 μ M in HBSS-Hepes buffer, pH 7.4 and added to the donor side, and HBSS-Hepes buffer, pH 7.4 is added to the receiver side. No pH gradient is used across the cell monolayer to minimize any proton-driven transport activity. The assay is incubated at 37°C for 60 minutes for the A-B assay and 40 minutes for the B-A assay. Samples are taken at time zero from the donor side and at the end of the incubation from both the donor and the receiver sides. Samples are analyzed by HPLC-MS/MS, and the P_{app} value, expressed as 10^{-6} cm/s, is calculated based on the appearance rate of the compound in the receiver side.

To evaluate the E ratio in the MDR1-MDCKII cells, it is important to compare the results to that from the parent, MDCKII cells, which are not transfected with the gene for MDR1. Possible P-gp substrates will have an E ratio for the MDR1-MDCKII cells that is at least two fold greater than the E ratio of the MDCKII parent cells.

To evaluate P-gp mediated efflux, verapamil, a known P-gp inhibitor, is included in the permeability assays. Since P-gp is an efflux transporter located on the apical side of the intestine, verapamil decreases the B-A permeability of a P-gp substrate. Verapamil may also result in increased A-B permeability since efflux of the compound is blocked resulting in more compound available to pass through to the B-side. In the presence of verapamil, the net flux ratio should approach unity or be significantly decreased from the E ratio in the absence of verapamil. However, overall permeability values should also be considered when determining whether absorption of a compound may be limited by permeability.

Colchicine and vinblastine are known P-gp substrates and are used as reference compounds in the permeability assays. Typical values for the reference compounds are shown in the table. Colchicine and vinblastine have significant E ratios in the absence of verapamil, and in the presence of verapamil, the E ratio is significantly decreased.

If a compound has an efflux ratio greater than two, and verapamil does not reduce the efflux ratio, then perhaps other efflux transporters are responsible for the secretion.

Typical permeability values (10^{-6} cm/s) for TC7 permeability (pH 7.4/7.4) assays ►

Drug	- verapamil (100 μ M)			+ verapamil (100 μ M)		
	A-B	B-A	E ratio	A-B	B-A	E ratio
colchicine	0.1	8.6	86	0.5	1.2	2.4
labetalol	9.1	42.1	4.6	39.5	21.9	0.6
propranolol	63.3	18.1	0.3	71.8	30.1	0.4
ranitidine	0.9	5.3	5.9	1.6	0.9	0.6
vinblastine	0.4	40.2	100	12.5	13.0	1.0

RECOVERY

Recovery of the test compound, expressed as percent, is provided. This is the amount of compound detected in both the receiver and the donor sides at the end of the assay relative to the amount detected in the donor side at time zero. If the recovery is dramatically different from 100%, the P_{app} value should be interpreted with caution. Low recovery is most likely due to nonspecific binding to the plastic and/or cells, retention within the cells, and/or degradation of the compound.

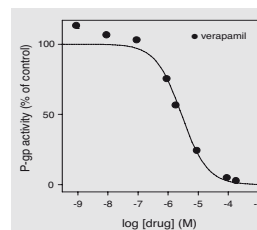
QUALITY CONTROL OF CELL MONOLAYERS

Fluorescein is used as the cell monolayer integrity marker. Fluorescein permeability assessment (in the A-B direction at pH 7.4) is performed after the permeability assay for the test compound. The cell monolayer that has a fluorescein permeability of less than 0.5, 1.5 and 2.5 ($\times 10^{-6}$ cm/s) for TC7, MDR1-MDCKII and MDCKII respectively, is considered intact.

P-gp INHIBITION ASSAYS

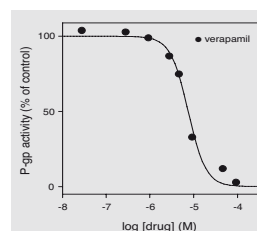
▶ P-gp INHIBITION WITH [³H]-DIGOXIN SUBSTRATE

Digoxin is a well-known substrate of P-gp. In this assay, a test compound is incubated with TC7 cells in both the A and the B sides at equal concentration at pH 7.4 in 96-well transwell plates. A trace amount of [³H]-digoxin is included in the B side, whereas unlabeled digoxin (10 μ M) is included in both the A and B sides. The incubation is allowed for 3 hours at 37°C with shaking, and samples from the A side are collected at the end of incubation and analyzed by liquid scintillation counting (Packard TopCount NXT). The percent of control P-gp activity is calculated by comparing the digoxin concentrations in the presence of test compound to that in the absence of the test compound. Multiple concentrations of test compound, typically from 0.01 μ M to 100 μ M, are tested to determine the IC₅₀ value. Verapamil is used as a positive control, which has an average IC₅₀ value of 5 μ M in this assay.



▶ P-gp INHIBITION WITH CALCEIN AM SUBSTRATE

Calcein AM is also a P-gp substrate. In this assay, the test compound is pre-incubated with the MDR1-MDCKII cells for 30 minutes at 37°C in 96-well plates, followed by addition of calcein AM at a final concentration of 2 μ M. The incubation is continued for another 30 minutes. Calcein AM, which is non-fluorescent, is believed to easily enter cells by passive diffusion. Once inside the cell, it is either transported out of the cell by P-gp, or hydrolyzed by intracellular esterases becoming a polar, fluorescent molecule that is trapped in the cell. The level of fluorescence is measured at the end of the incubation, and the fluorescence reading indicates retention of calcein within the cells. A higher fluorescence reading represents more calcein within the cells due to greater inhibition of P-gp activity. The percent inhibition of the P-gp activity is calculated relative to the percent inhibition obtained with 100 μ M verapamil. The fluorescence reading obtained in the presence of 100 μ M verapamil is considered 100% inhibition of the P-gp activity. Typically, multiple concentrations of a test compound are assayed to determine an IC₅₀ value. Verapamil has an average IC₅₀ value of 5 μ M in this assay.



CUSTOMIZATION

All assays can be customized as per the customer's specifications.

DRUG DEVELOPMENT

P-gp-MEDIATED DRUG-DRUG INTERACTION PROFILE

The FDA draft guidance for industry on drug interaction studies addresses study design and data analysis for evaluating P-gp substrates and inhibitors¹. It recommends the bi-directional transport assay as the definitive assay for identifying P-gp substrates and inhibitors since this measures drug efflux in a more direct manner than other methods.

The P-gp-mediated drug-drug interaction profile is a set of assays designed to comply with the Draft guidance recommendations.

All assays in this profile are performed with 24-well transwell plates, and this profile is intended to support drug development.

■ P-gp SUBSTRATE EVALUATION

Compounds are first evaluated in the bi-directional permeability assays (A-B and B-A in the TC7 cell line), for a single concentration and multiple time points, as a preliminary assessment to evaluate the linear flux of the test compound. The A-B permeability of the compound in a blank filter (no cell monolayer) is also included in this step to assess possible non-specific binding of the test compound to assay plate. The test compound is further evaluated in the permeability assays in the presence of P-gp inhibitors to evaluate P-gp mediated efflux. The P-gp inhibitors verapamil and ketoconazole are included in the permeability assays, and the well-known P-gp substrate, colchicine, is used as a positive control. Also, the test compound is assayed at three concentrations, to examine concentration-dependent efflux. The permeability values efflux ratios and recovery are reported.

■ P-gp INHIBITOR EVALUATION

Test compounds are incubated with TC7 cells and [³H]-digoxin is used as the probe P-gp substrate. The permeability of [³H]-digoxin is determined in both the A-B and B-A directions in the presence and absence of the test compound. The test compound, when present, is included in both the A and B sides at equal concentrations. Concentrations of [³H]-digoxin are determined using liquid scintillation counting. Seven test concentrations are evaluated, and the IC₅₀ value for the test compound is determined.

¹ FDA (2006) Drug Interaction Studies - Study Design, Data Analysis, and Implications for Dosing and Labeling



FRANCE
Le Bois l'Évêque
86600 CELLE L'ÉVESCAULT
tel. +33 (0)5 49 89 30 00

(Headquarters)
155 boulevard Haussmann
75008 PARIS
tel. +33 (0)1 45 64 44 60

USA
15318 N.E. 95th Street
REDMOND, WA 98052
tel. +1 (425) 895 8666

JAPAN
Namiki Shoji Co., Ltd.
Kenseishinjuku Bldg. 5-5-3
Shinjuku, Shinjuku-ku
TOKYO, 160-0022
tel. +81 (0)3 3354 4026
fax +81 (0)3 3352 2196

CHINA
Ai Di Sheng (Edison) Road 326,
302-1 room
Zhangjiang High-Tech Park
SHANGHAI
tel. +86 18702160370

■ QUESTIONS OR CONCERNS?

Please contact us: sales@cerep.com