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## P-GP DRUG INTERACTION ASSAY KIT

catalogue # D05001 - 96 wells

This assay has been developed and validated together with SPI-BIO and the CEA.

*For research laboratory use only. Not for human diagnostic use.*

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## P-GP DRUG INTERACTION ASSAY KIT

96-well - Storage: -20°C / -80°C\*  
Expiry date: stated on the package

This kit contains:

- ☞ One 96-well plate,
- ☞ One vial of verapamil (600 µM in water),
- ☞ One vial of progesterone (1.2 µM in 30% ethanol),
- ☞ One vial of vinblastine (100 µM in water),
- ☞ One vial of pyruvate kinase & lactate dehydrogenase,
- ☞ One vial of phosphoenolpyruvate,
- ☞ One vial of NADH,
- ☞ One vial of MgATP,
- ☞ One vial of non-specific ATPase inhibitors,
- ☞ One vial of enzymatic buffer,
- ☞ One instruction booklet,
- ☞ One template sheet.



\*☞ Two vials of P-gp containing membrane vesicles that are **packed separately** and to be **stored at -80°C**.

Each kit contains sufficient reagents for 96 wells. The number drug to be evaluated depends on the design of the study. Twenty compounds may be assayed at 4 different concentrations with or without verapamil, progesterone or vinblastine (three possible reference compounds). In duplicate, 10 compounds may be evaluated.

## PRECAUTIONS FOR USE

**Users are recommended to read all instructions for use before starting work.**

Each time a new pipet tip is used, aspirate a reagent and dispense it back into the same vessel. Repeat this operation two or three times before distribution.

For research laboratory use only.

Not for human diagnostic use.

Do not pipet liquids by mouth.

Do not use kit components beyond the expiration date.

Do not eat, drink or smoke in area in which kit reagents are handled.

Avoid splashing.

## INTEREST AND PRINCIPLE OF THE ASSAY

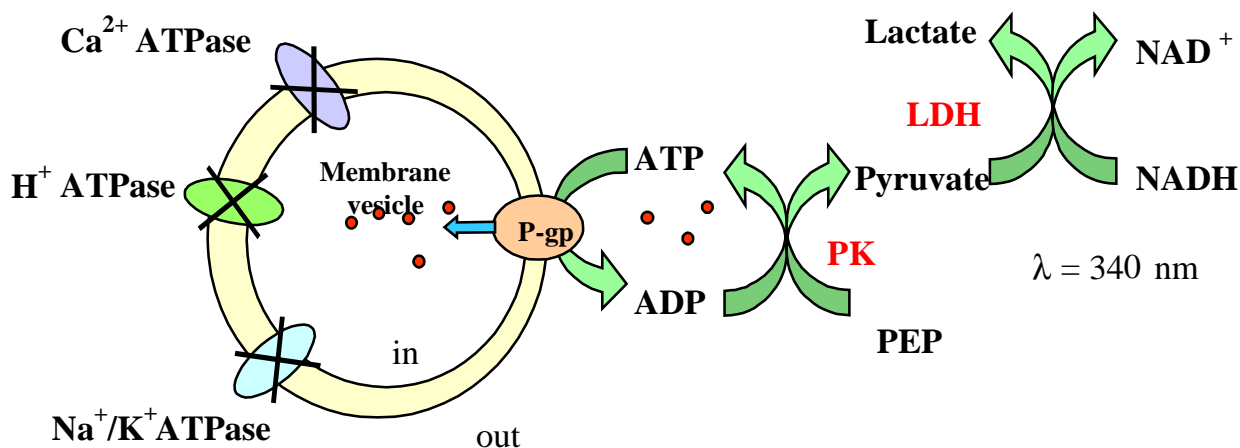
P-Glycoprotein (P-gp) is an active plasma membrane transporter involved in drug pharmacokinetics and cellular detoxification. P-gp exhibits a high drug-dependent ATP hydrolysis activity that is a reflection of its drug transport ability. The test of drug stimulation or inhibition of ATPase activity may be used to screen the potential drug interaction with P-gp.

This assay allows an *in vitro* screening for testing drug interaction with P-gp, based on the study of modulation of basal or induced ATPase activity from enriched P-gp membrane vesicle preparation. P-gp ATPase activity is measured by a spectrophotometric method based on continuous monitoring of ADP formation, in the vesicle suspension medium.

The basal ATPase activity of P-gp is defined as its MgATP hydrolysis activity determined in the absence of any added drug. Modulation of basal ATPase activity is performed by adding various drugs at different concentrations.

Some compounds may modulate stimulated activity of P-gp. This kit permits to assess to evaluate the modulation of stimulated P-gp activity by three different compounds (verapamil, progesterone or vinblastine) that interact on different P-gp sites and are known to be a P-gp Substrate.

Data are analysed by comparing the variation of the basal or stimulated activities to the reference activities. Tests are performed on 96-well microtiter plate. The principle of the assay is summarised below:



PK : pyruvate kinase - LDH : lactate dehydrogenase - PEP : phosphoenolpyruvate

## FREQUENTLY ASKED QUESTIONS

Prior reading the assay procedure, we recommend to read the answers to the following questions. This will help the users to understand better this assay and to understand the meaning of the obtained data.

### WHERE DO THE MEMBRANES COME FROM?

Our P-gp containing membranes are prepared from highly resistant MDR cells, the DC-3F/ADX line, which are Chinese Hamster lung fibroblasts, spontaneously transformed and selected from the parent sensitive cell line DC3-F by stepwise selection against increasing concentrations of actinomycin D.

### ARE YOU SURE IT OVEREXPRESSES THE P-GP AND NOT OTHER ABC TRANSPORTERS?

P-gp is highly overexpressed in the DC-3F/ADX cells, where it represents about 15% of the total membrane proteins, compared to the parent DC-3F cells where it cannot be detected by the C219 or C494 antibodies specific to P-gp. In addition, P-gp is the only membrane protein so overexpressed, as evidenced by SDS-PAGE which shows only one major band at 150-160 kDa, and as shown by a densitometric analysis of the electrophoresis gel. Thus, it cannot be excluded that other ABC proteins, as well as other membrane ATPases, are present in our vesicles but their amount are limited to low expression levels as found in the parent DC-3F cells. Control experiments using membrane vesicles prepared from the DC-3F cells can be performed to ensure the role of P-gp in the ATPase signal observed on the vesicles from DC-3F/ADX. It has been published in: Garrigos M. *et al.*

### WHAT IS THE TYPICAL BASAL SIGNAL AND WHAT IS THE TYPICAL DRUG-MEDIATED RESPONSE?

The typical level of basal ATPase activity is somehow dependent on the batch of membranes used, but it is generally within the range from 60 to 150 nmol/min/mg total membrane. The maximum drug-mediated response is an activation of ATPase activity by a factor of 2 observed for verapamil at 30μM.



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#### **IS THE KIT ABLE TO DISTINGUISH SUBSTRATE FROM INHIBITOR?**

The test can not answer accurately to the question. Classification of drugs in inhibitors or substrates requires the use of monolayer efflux systems (Caco2 or MDR transfected cells). The comparison of cells systems and membrane assays have been recently described (Adachi *et al.*; Polli *et al.*). As far as these papers and our own study is concerned (Orlowski *et al.*) it results that all compounds positive in the ATPase assay are either substrate or inhibitor.

- If you obtain a minimum of 30% increase or decrease of the basal or the stimulated activity of P-gp, you can conclude that your compound interacts with P-gp.
- If your molecule does not show interaction, it is not transported by P-gp (neither substrate nor inhibitor).
- If you obtain a signal close to 0, you identify an inhibitor that blocks the ATP hydrolysis by irreversible binding on the nucleotide site.

The advantage of this assay is to provide information about specific interaction (binding) between P-gp and tested molecules in a more simple manner than using transport tests performed on living cells (Caco-2, MDCK, etc..) This assay avoids using any radio-labelled compounds or specific techniques such as HPLC-MS/MS to measure each drug. Obviously, this assay should be considered as a first step screening performed on a number of candidate molecules. It should be completed for some selected drugs by a direct transport assay.

#### **WHY DETERMINING THE INTERACTION BY MEASURING BASAL AND STIMULATED ACTIVITIES OF P-GP?**

Among the number of compounds interacting with P-gp, it has been demonstrated that some of them do not change the basal ATPase activity level. However, these compounds do alter the ATPase activity stimulated by other known substrates, such as verapamil, progesterone and vinblastine, revealing their interaction with P-gp.

#### **WHY ARE YOU USING THREE DIFFERENT COMPOUNDS?**

The P-gp is described to have different binding sites for the various drugs it recognizes. We have chosen verapamil, progesterone and vinblastine because these drugs bind to distinct binding sites, and thus can "sensor" with a high probability the interaction of a number of other ligands on P-gp.

It should be pointed out that classical ATPase tests are not discriminatory since they do not use the ability of a drug to modulate the ATPase activity in presence of one specific drug, (verapamil, progesterone and vinblastine ). We found that our approach avoids false negative compounds.

#### **WHAT ARE THE CORRECT VALUES WE MAY OBTAIN?**

The NSA should be under 1.5 mAU/min, the ratio BA/NSA higher than 2. The ratio TA/BA should be higher than 2.

The relative activity of Vinblastine, Verapamil, Progesterone should be close to 1.4, 3, 1.7 respectively.

#### **IN THE CALCULATION, WHERE DOES THE 26 10<sup>-2</sup> VALUE COME FROM?**

This figure comes from the Beer-Lambert law ( $A = \epsilon \times L \times C$ ). Precisely:

$$\text{Activity(nmol/mg/min)} = \text{AU(mAU/min)} \times 26 \times 10^{-2} \times \text{protein concentration}^{-1} \Leftrightarrow$$

$$\text{Activity(nmol/mg/min)} = \text{AU(mAU/min)} \times 1000 / [6230 \text{ (NADH extinction coefficient in } \text{mmol}^{-1} \cdot \text{L} \cdot \text{cm}^{-1}) \times 0.62 \text{ (optical pathway in cm)}] \times \text{protein concentration}^{-1} \text{ (mg/ml)}.$$

#### **WHAT SOLVENT COULD I USE FOR THE COMPOUNDS I'D LIKE TO TEST?**

The organic solvent should be lower than 1%, and DMSO lower than 0.5%. We often choose Ethanol.



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## MATERIALS AND EQUIPMENT REQUIRED

In addition to standard laboratory equipment, the following material is required:

- ☞ Precision micropipettes (20, 200 & 1000  $\mu$ L) with corresponding tips
- ☞ Multipipettor with 0.5 and 1.0 mL combitips
- ☞ Multichannel pipettors 5-50  $\mu$ L and 50-300  $\mu$ L
- ☞ Spectrophotometer plate reader (340 nm filter)
- ☞ Microplate incubator at 37°C
- ☞ Microplate shaker
- ☞ Distilled or deionized water
- ☞ Polypropylene tubes

## ASSAY PROCEDURE

It is recommended to perform the assays in duplicate and to follow the instructions hereafter.

The 96-well plate and reagents are provided ready to use. After use, store immediately all reagents at -20°C. Use different tips to pipet the various reagents.

### PRECAUTION FOR OPTIMAL USE

- ☞ Turn on or switch on the spectrophotometer and the incubator at 37°C,
- ☞ Distribute samples with multichannel pipettor to keep plate at 37°C as much as possible,
- ☞ Final organic solvent concentration must be below 1% in each well.

### PIPETTING THE REAGENTS

All samples and reagents must reach +4°C prior to distribution. Maintain all reagents in the ice bath during use.

### DISTRIBUTION OF REAGENTS AND SAMPLES

A plate set-up is suggested below. The contents of each well may be recorded on the sheet provided with the kit.

	1	2	3	4	5	6	7	8	9	10	11	12
<b>A</b>	Blank	Vinblastin	Drug 2	Drug 6	Drug 1	Drug 1	Drug 18	Drug 22	Drug 26	Drug 30	Drug 3	Drug 3
<b>B</b>	Blank	Vinblastin	Drug 2	Drug 6	Drug 10	Drug 1	Drug 18	Drug 22	Drug 2	Drug 30	Drug 34	Drug 3
<b>C</b>	Non Activit	Verapami	Drug 3	Drug 7	Drug 1	Drug 15	Drug 1	Drug 23	Drug 27	Drug 31	Drug 3	Drug 3
<b>D</b>	Non Activit	Verapami	Drug 3	Drug 7	Drug 1	Drug 15	Drug 19	Drug 2	Drug 27	Drug 31	Drug 35	Drug 3
<b>E</b>	Total	Progesterone	Drug 4	Drug 8	Drug 1	Drug 16	Drug 2	Drug 2	Drug 28	Drug 32	Drug 36	Drug 4
<b>F</b>	Total	Progesteron	Drug 4	Drug 8	Drug 12	Drug 16	Drug 20	Drug 2	Drug 28	Drug 32	Drug 3	Drug 4
<b>G</b>	Basa Activit	Drug 1	Drug 5	Drug 9	Drug 13	Drug 1	Drug 21	Drug 2	Drug 29	Drug 33	Drug 37	Drug 4
<b>H</b>	Basa Activit	Drug 1	Drug 5	Drug 9	Drug 1	Drug 17	Drug 2	Drug 2	Drug 29	Drug 33	Drug 37	Drug 4

**Blank:** signal linked to enzymatic buffer only

**Non Specific activity:** signal measured in absence of membrane vesicles

**Total activity:** signal corresponding non specific ATPase activity

**Basal activity:** signal of basal P-gp activity

**Verapamil, progesterone, vinblastine:** signal of stimulated P-gp activity by these compounds



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- ↳ Prepare the compounds to be tested at the correct concentrations (e.g. 500, 50, 5, and 0.5  $\mu\text{M}$ ). In this case the final concentration will be 50, 5, 0.5 and 0.05  $\mu\text{M}$ .
- ↳ Distribute in Blank wells: 200  $\mu\text{L}$  enzymatic buffer
- ↳ Distribute with multipipettor in each other wells:
  - 80  $\mu\text{L}$  of enzymatic buffer,
  - 20  $\mu\text{L}$  of PK/LDH solution,
  - 10  $\mu\text{L}$  of PEP solution,
  - 10  $\mu\text{L}$  of NADH solution.
- ↳ Dispense in total activity wells:
  - 60  $\mu\text{L}$  of enzymatic buffer.
- ↳ Distribute in basal activity wells:
  - 30  $\mu\text{L}$  of Non-specific ATPase inhibitor solution,
  - 30  $\mu\text{L}$  of enzymatic buffer.
- ↳ Distribute in reference compound wells:
  - 30  $\mu\text{L}$  of Non-specific ATPase inhibitor solution,
  - 10  $\mu\text{L}$  of reference compound (verapamil 30  $\mu\text{M}$ , progesterone 60  $\mu\text{M}$ , or vinblastine 5  $\mu\text{M}$ ), 20  $\mu\text{L}$  of enzymatic buffer.
- ↳ Distribute in Non-specific activity wells:
  - 30  $\mu\text{L}$  of Non-specific ATPase inhibitor solution, 30  $\mu\text{L}$  of enzymatic buffer.
- ↳ Distribute in all the other wells:
  - 30  $\mu\text{L}$  Non-specific ATPase inhibitor solution,
  - 10  $\mu\text{L}$  of reference compound or enzymatic buffer to test compound on basal activity.

#### **INCUBATING, DEVELOPING AND READING THE PLATE**

- ↳ Shake the plate 10 seconds and read the plate at 340 nm to verify NADH absorbance in each well.
- ↳ Incubate the 96-well plate for 30 minutes at 37°C.
- ↳ Add:
  - 10  $\mu\text{L}$  of enzymatic buffer in Non specific activity wells,
  - 10  $\mu\text{L}$  membrane vesicles in all the other wells except the Blank wells. Just before distribution, vortex membrane vesicles to homogenate suspension and eliminate aggregates.
- ↳ incubate the 96-well plate for 5 minutes at 37°C.
- ↳ Dispense 20  $\mu\text{L}$  of compound at each tested concentration & incubate for 5 minutes at 37°C.
- ↳ Dispense 10  $\mu\text{L}$  of MgATP in every well but blank wells & incubate the plate for 20 minutes at 37°C.
- ↳ Shake the plate 10 seconds & read the plate at 340 nm. Then keep on incubating the plate & read 20 minutes later.



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Assay protocol - one concentration						
Steps	Blank	Non Specific Activity	Total Activity	Basal Activity	Reference compound	Samples
Step 1: distribute,						
Enzymatic buffer	200 µL	80 µL	80 µL	80 µL	80 µL	80 µL
PK/LDH solution		20 µL	20 µL	20 µL	20 µL	20 µL
PEP solution		10 µL	10 µL	10 µL	10 µL	10 µL
NADH solution		10 µL	10 µL	10 µL	10 µL	10 µL
Enzymatic buffer*		30 µL	60 µL	30 µL	20 µL	10 µL*
Non-specific ATPase inhibitor solution		30 µL		30 µL	30 µL	30 µL
Reference compound*					10 µL	10 µL*
Step 2:	Shake the plate 10 seconds & read the plate at 340 nm.					
Step 3:	Incubate the 96-well plate for 30 minutes at 37°C.					
Step 4: add,						
Enzymatic buffer		10 µL				
Membrane vesicles			10 µL	10 µL	10 µL	10 µL
Step 5:	Incubate the 96-well plate for 5 minutes at 37°C.					
Step 6: dispense,						
tested compound						20 µL
Step 7:	Incubate the 96-well plate for 5 minutes at 37°C.					
Step 8: dispense,						
MgATP		10 µL	10 µL	10 µL	10 µL	10 µL
Step 9:	Incubate the 96-well plate for 20 minutes at 37°C.					
Step 10:	Shake the plate 10 seconds & read the plate at 340 nm (t0). Keep on incubating the plate & read 20 minutes later (t20).					
Final volume	200 µL	200 µL	200 µL	200 µL	200 µL	200 µL

\*: you may either test your compound versus the basal activity. If so, add 10 µL of enzymatic buffer. If you wish to test your compound with a reference compound add 10 µL of this reference.

#### MEASUREMENT AT VARIOUS CONCENTRATIONS

It may be possible to assess P-gp drug interaction at various concentrations. To do so,

- ↳ Add 5 µL of the drug at the lowest concentration (e.g. 0.05 µM, final concentration) & incubate for 5 minutes at 37°C. Dispense then 10 µL of MgATP in every wells but blank wells & incubate the plate for 20 minutes at 37°C. Shake the plate 10 seconds & read the plate at 340 nm. Then, keep on incubating the plate & read 20 minutes later,
- ↳ Add 5 µL of the drug at a second concentration (e.g. 0.5 µM, final concentration), shake the plate 10 seconds & read the plate immediately and 20 minutes later,
- ↳ Add 5 µL of the drug at a third concentration (e.g. 5 µM, final concentration), shake the plate 10 seconds & read the plate immediately and 20 minutes later,
- ↳ Add 5 µL of the drug at a fourth concentration (e.g. 50 µM, final concentration), incubate 10 minutes at 37°C, shake the plate & read the plate immediately and 20 minutes later.

Make sure to take into account the final volume to determine the final concentration of the compound. For example:

- ↳ Add 5 µL of a compound at 1.9 µM for a 0.05 µM final concentration,
- ↳ Add 5 µL of a compound at 17.2 µM for a 0.5 µM final concentration,
- ↳ Add 5 µL of a compound at 176 µM for a 5 µM final concentration,
- ↳ Add 5 µL of a compound at 1.79 mM for a 50 µM final concentration.



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Alternative assay protocol - four concentrations						
Steps 1 to 5:	See above (remain unchanged)					
Step 6: dispense, tested compound (first concentration)						5 $\mu$ L
Steps 7 to 10:	See above (remain unchanged)					
Step 11: dispense, tested compound (second concentration)						5 $\mu$ L
Step 12:	Shake the plate 10 seconds & read the plate at 340 nm. Keep on incubating the plate & read 20 minutes later.					
Step 13: dispense, tested compound (third concentration)						5 $\mu$ L
Step 14:	Shake the plate 10 seconds & read the plate at 340 nm. Keep on incubating the plate & read 20 minutes later.					
Step 15: dispense, tested compound (fourth concentration)						5 $\mu$ L
Step 16:	Shake the plate 10 seconds & read the plate at 340 nm. Keep on incubating the plate & read 20 minutes later.					
Final volume	200 $\mu$ L	200 $\mu$ L	200 $\mu$ L	200 $\mu$ L	200 $\mu$ L	200 $\mu$ L

### DATA ANALYSIS

Make sure that your plate reader has subtracted the absorbance readings of the blank wells from the absorbance readings of the rest of the plate. If not, do it now.

- ↳ Calculate the average absorbance, the standard deviation and the coefficient of variation for each duplicate.
- ↳ On the mean value, determine the  $\Delta$ mAU/minute between 0 and 20 minutes, for each drug concentration. Calculate the corresponding activity (nmol/mg/min):  $(\Delta$ mAU/minute  $\times$  26  $10^{-2}$ ) / protein concentration (see leaflet enclosed for its value).
- ↳ Subtract the non specific activity (NSA) of wells without vesicle from each other wells.
- ↳ Calculate relative activity of each compound concentration compared to the reference activity (basal or stimulated activity).

For example (reference: basal activity):

- Cyclosporin A (50  $\mu$ M): 130.1 nmol/mg/min
- Verapamil (30  $\mu$ M): 393.5 nmol/mg/min
- Basal activity: 159.4 nmol/mg/min

Cyclosporin A relative activity:  $130.1/159.4 = 0.81$

Verapamil relative activity:  $393.5/159.4 = 2.45$





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## TYPICAL DATA

Compound	Relative activities															
	Basale activity				VRP 30 $\mu$ M				PRG 60 $\mu$ M				VBL 5 $\mu$ M			
	0.05 $\mu$ M	0.5 $\mu$ M	5 $\mu$ M	50 $\mu$ M	0.05 $\mu$ M	0.5 $\mu$ M	5 $\mu$ M	50 $\mu$ M	0.05 $\mu$ M	0.5 $\mu$ M	5 $\mu$ M	50 $\mu$ M	0.05 $\mu$ M	0.5 $\mu$ M	5 $\mu$ M	50 $\mu$ M
5-fluorouracil	0.92	0.91	0.92	0.96	1.01	1.00	1.00	1.02	0.94	0.97	0.90	0.82	1.06	1.05	1.03	1.03
Actinomycine D	1.28	1.19	0.75	0.87	0.94	0.93	0.74	0.42	0.93	0.89	0.66	0.63	0.91	0.88	0.90	1.13
Amiloride	0.92	1.15	1.10	1.19	0.99	1.05	0.97	1.04	0.96	0.92	1.01	1.02	0.96	0.97	0.96	1.12
Bromocriptine	0.86	0.59	0.42	0.26	0.78	0.66	0.22	NT	0.66	0.38	0.32	NT	NT	NT	NT	NT
Camptothecin	0.90	0.92	0.98	0.68	0.88	0.94	0.92	0.86	0.91	0.92	0.91	0.78	0.83	0.90	0.82	0.32
Chaps	0.92	1.01	1.01	0.99	0.89	0.96	0.97	0.96	0.92	0.94	0.94	0.90	0.96	0.95	1.01	1.08
Chromomycin A3	1.01	1.04	2.02	0.86	0.95	0.89	0.95	0.99	0.90	0.91	0.91	0.67	0.91	0.91	1.41	1.05
Cis-Platinium	0.95	0.90	1.04	0.98	0.98	1.03	0.98	1.02	0.90	0.89	0.91	0.82	0.90	0.92	0.94	0.95
Colchicine	0.94	1.06	1.10	1.33	0.86	0.95	0.97	0.94	1.02	1.00	1.02	1.04	1.12	1.18	1.21	1.15
Daunomycine	0.91	0.94	0.96	0.75	0.92	0.97	0.98	0.94	0.94	0.93	0.87	0.68	0.84	0.95	0.99	0.87
Dipyridamole	0.95	1.23	2.01	2.10	1.00	0.99	0.98	0.83	1.04	1.23	1.62	1.36	0.93	0.95	1.06	1.53
Emetine	1.10	1.47	2.55	2.78	0.99	0.96	0.84	0.65	0.99	0.97	1.05	1.01	0.93	1.00	1.14	1.65
Ivermectine	0.83	0.62	0.32	0.19	0.98	0.69	0.22	0.14	0.91	0.63	0.31	0.21	0.90	0.64	0.35	0.28
Methotrexate	1.09	1.08	1.02	1.09	1.04	1.04	0.96	1.05	0.95	0.94	0.94	0.95	0.93	0.96	0.93	0.91
Midazolam	0.90	0.94	1.19	1.54	0.88	0.95	0.93	0.71	1.02	1.09	1.05	0.88	1.07	1.13	1.03	0.64
Naproxen	0.94	0.94	0.94	0.97	0.89	0.98	1.00	1.02	0.97	1.05	1.07	1.05	1.13	1.14	1.20	1.18
Paclitaxel	1.00	0.96	1.42	2.39	1.00	0.95	0.97	0.99	0.89	0.95	0.93	1.01	0.96	0.96	0.97	1.03
Probenecid	1.00	1.01	1.02	1.06	1.05	1.03	1.00	1.07	0.99	1.03	1.01	0.95	0.99	1.04	1.05	1.02
Progesterone	1.00	1.11	1.42	2.39	0.93	0.98	0.95	0.56	NT	NT	NT	NT	0.99	1.02	0.88	0.68
Puromycin	0.97	0.99	1.04	1.52	0.97	0.98	1.01	1.08	0.94	1.01	0.96	0.92	0.89	0.91	0.93	1.00
Quercetine	1.06	1.10	0.92	1.19	0.98	0.97	0.82	0.88	0.88	0.93	0.77	0.53	0.89	0.90	0.90	0.87
Quinidine	0.99	1.15	1.90	2.11	1.05	1.02	0.95	0.80	0.98	0.99	0.95	0.98	1.01	1.05	1.06	1.22
Rapamycin	1.02	1.27	0.69	0.40	0.92	0.73	0.23	0.11	0.90	0.56	0.24	0.10	0.98	0.80	0.41	0.33
Sucrose	0.84	0.89	0.91	1.01	0.93	0.97	1.00	1.04	0.92	0.93	0.94	0.93	1.02	0.97	1.01	1.13
Tamoxifen	1.02	1.16	0.73	0.51	0.96	0.88	0.60	0.30	0.87	0.90	0.37	0.34	0.87	0.73	0.48	0.49
Trifluoperazine	1.06	1.34	1.59	0.74	1.02	0.97	0.81	0.43	0.89	0.82	0.69	0.13	0.92	0.82	0.62	0.44
Verapamil	1.11	2.01	2.80	2.86	NT	NT	NT	NT	NT	0.73	0.55	0.45	NT	NT	NT	NT
Vinblastin	0.98	1.37	1.21	0.32	0.93	0.91	0.56	0.12	0.93	0.65	0.30	0.11	NT	NT	NT	NT

NT : not tested

## ASSAY VALIDATION & CHARACTERISTICS

### INTRA & INTER-ASSAY VARIATION

	Intra-assay variation (n=8)	
	Activity (nmol/mg/min)	Relative activity*
Basal activity	213.3 $\pm$ 21.0 (9.8%)	1.00
Verapamil (30 $\mu$ M)	498.2 $\pm$ 19.2 (3.9%)	2.34 $\pm$ 0.09 (3.9%)
Progesterone (50 $\mu$ M)	383.1 $\pm$ 8.8 (2.3%)	1.80 $\pm$ 0.04 (2.3%)
Cyclosporin A (50 $\mu$ M)	150.2 $\pm$ 19.4 (12.9%)	0.70 $\pm$ 0.09 (12.9%)
Vinblastine (50 $\mu$ M)	61.8 $\pm$ 7.1 (11.4%)	0.29 $\pm$ 0.03 (11.4%)

Inter-assay variation (n=4)		
	Activity (nmol/mg/min)	Relative activity*
Basal activity	171.1 ± 30.8 (18.0%)	1.00
Verapamil (30 µM)	449.4 ± 73.0 (16.2%)	2.64 ± 0.17 (6.6%)
Progesterone (50 µM)	323.2 ± 58.0 (17.9%)	1.89 ± 0.11 (5.9%)
Cyclosporin A (50 µM)	147.0 ± 35.5 (24.1%)	0.85 ± 0.06 (7.2%)
Vinblastine (50 µM)	53.3 ± 18.4 (34.6%)	0.31 ± 0.07 (21.3%)

Inter-vesicular variation (n=4)		
	Activity (nmol/mg/min)	Relative activity*
Basal activity	159.4 ± 30.4 (19.1%)	1.00
Verapamil (30 µM)	393.5 ± 102.7 (26.1%)	2.45 ± 0.42 (17.2%)
Progesterone (50 µM)	305.0 ± 56.1 (18.4%)	1.92 ± 0.20 (10.2%)
Cyclosporin A (50 µM)	130.1 ± 29.5 (22.7%)	0.81 ± 0.08 (9.4%)
Vinblastine (50 µM)	39.0 ± 8.0 (20.5%)	0.25 ± 0.02 (9.4%)

\* Relative activities are compared to the basal activity

#### VALIDATION

It has been evidenced that the P-gp ATPase test correlates at 90 % for 29 compounds known from the literature for their positive (+) or negative (-) interaction with P-gp. Compounds were positive (+) in 96-well microplates for a variability >30% compared with reference activity.

Litterature data	+	Amiloride Probenecid	Actinomycine D Bromocriptine Camptothecin Chromomycine A3 Colchicine Daunomycine Dipyridamole Emetine Ivermectine Midazolam	Paclitaxel Progesterone Puromycine Quercetine Quinidine Rapamycin Tamoxifen Trifluoperazine Verapamil Vinblastine
	-	5-fluorouracil Chaps Cis-platinum Methotrexate Naproxen Sucrose	Trypan blue	
		-	+	
		Modulation of ATPase activities		



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## ASSAY TROUBLE SHOOTING

- ☞ Low activity: no vesicle or no MgATP in well, or P-gp degradation.
- ☞ All activities are low: verify that incubator temperature is at 37°C.
- ☞ Blank value too high: presence of NADH oxydant substance.
- ☞ High dispersion of duplicates: poor pipetting technique or low vortex of membrane vesicle suspensions.

These are a few examples of trouble shooting that may occur. If you need further explanation, SPI-BIO will be happy to answer any question or information about this assay. Please feel free to contact our technical support staff by letter, phone (33 (0)1 39 30 62 60), fax (33 (0)1 39 30 62 99) or E-mail (contact@spibio.com), and be sure to indicate the lot number of the kit (see outside the box).

SPI-BIO proposes a training workshop. For further information, please contact our Customer Relation Representative by letter, phone (33 (0)1 39 30 62 60), fax (33 (0)1 39 30 62 99) or E-mail (contact@spibio.com).

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