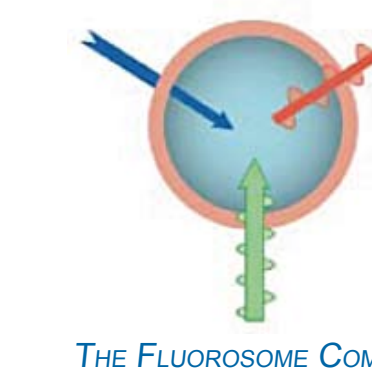


A Fast and Simple Method for Measuring P-Glycoprotein (Pgp) Inhibition The Fluorosome[®]-*trans*-pgp Assay and the FLUOstar Omega



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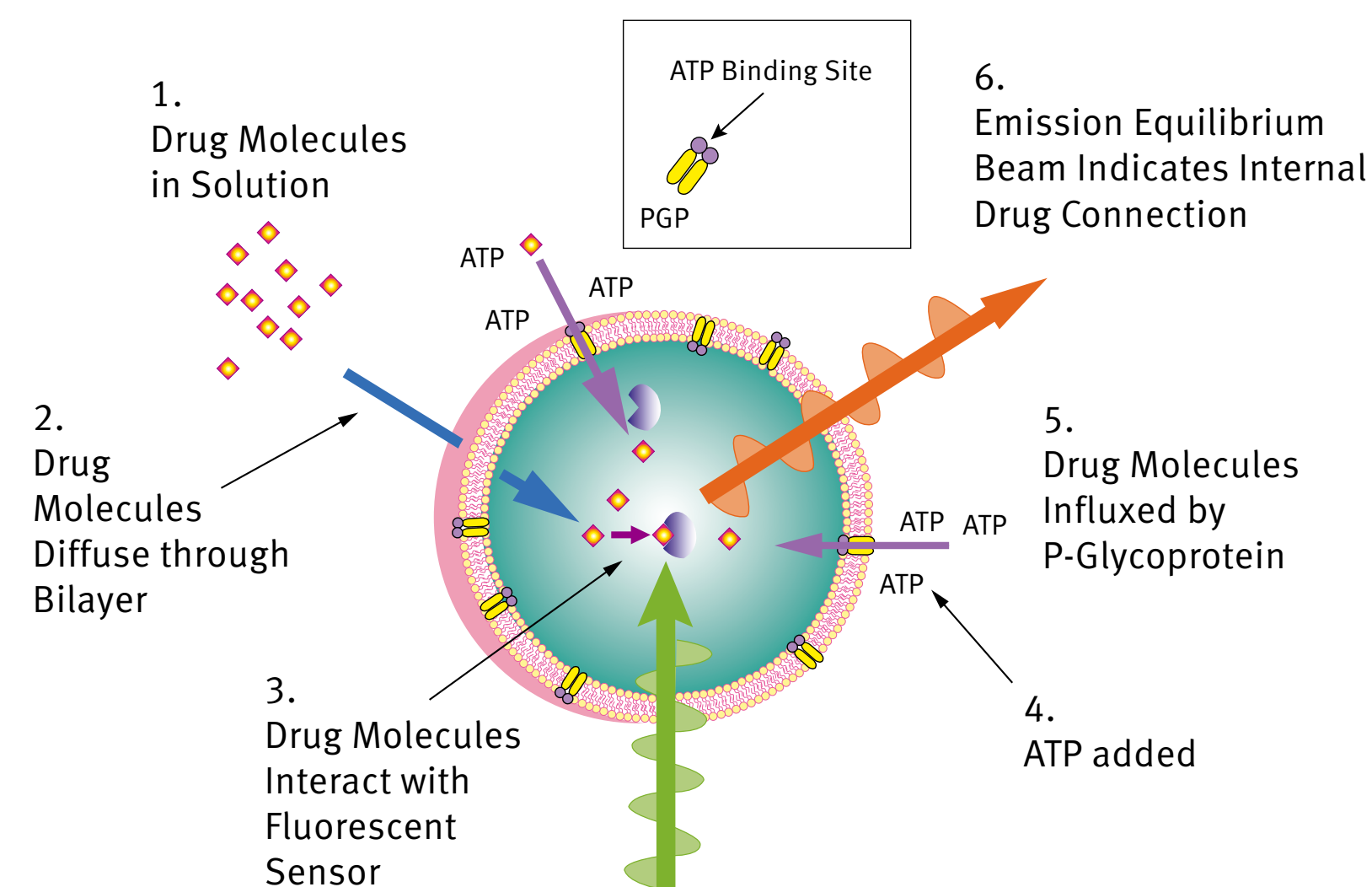
Introduction

P-glycoprotein (Pgp; ABCB1), a member of the ATP-binding cassette (ABC) superfamily, exports structurally diverse hydrophobic compounds from the cell driven by ATP hydrolysis. Pgp expression has been linked to the efflux of chemotherapeutic drugs in human cancers leading to multidrug resistance. Pgp activity can also result in low oral absorption and poor brain penetration. Interaction of drugs with the Pgp may also cause an increase in toxicity of co-administered compounds.

Interaction of drugs with active transporters such as Pgp is of increasing interest to the pharmaceutical industry based, in large part, on new draft FDA guidelines requiring knowledge of whether a drug candidate is a substrate and/or inhibitor of Pgp.

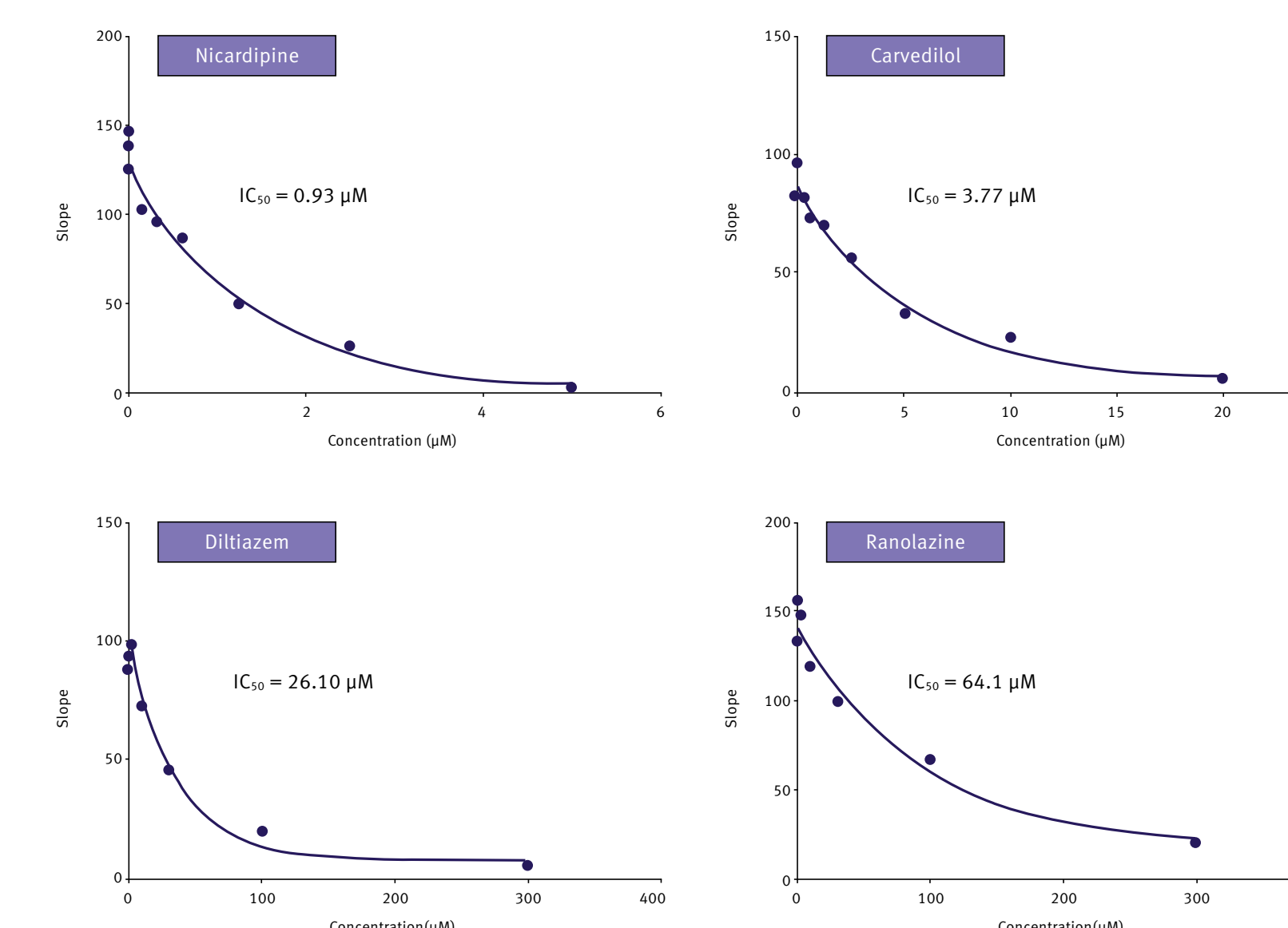
The Fluorosome Company's Fluorosome[®]-*trans*-pgp assay, together with BMG LABTECH's FLUOstar Omega, provides a rapid, sensitive and specific reconstituted Pgp liposome assay system for identification of compounds that interact with the transporter. The assay measures the ability of a compound to compete with a Pgp substrate for transport, and determines the IC₅₀ value. The FLUOstar Omega's ability to inject at the point of measurement assures that no data is lost. Up to 50 readings per second taken concurrently upon injection, provides ample data for calculation of first order transport rates. Single concentration inhibition measurements can be made very rapidly, and an IC₅₀ determination carried out in only 10 minutes thereby.

Assay Principle

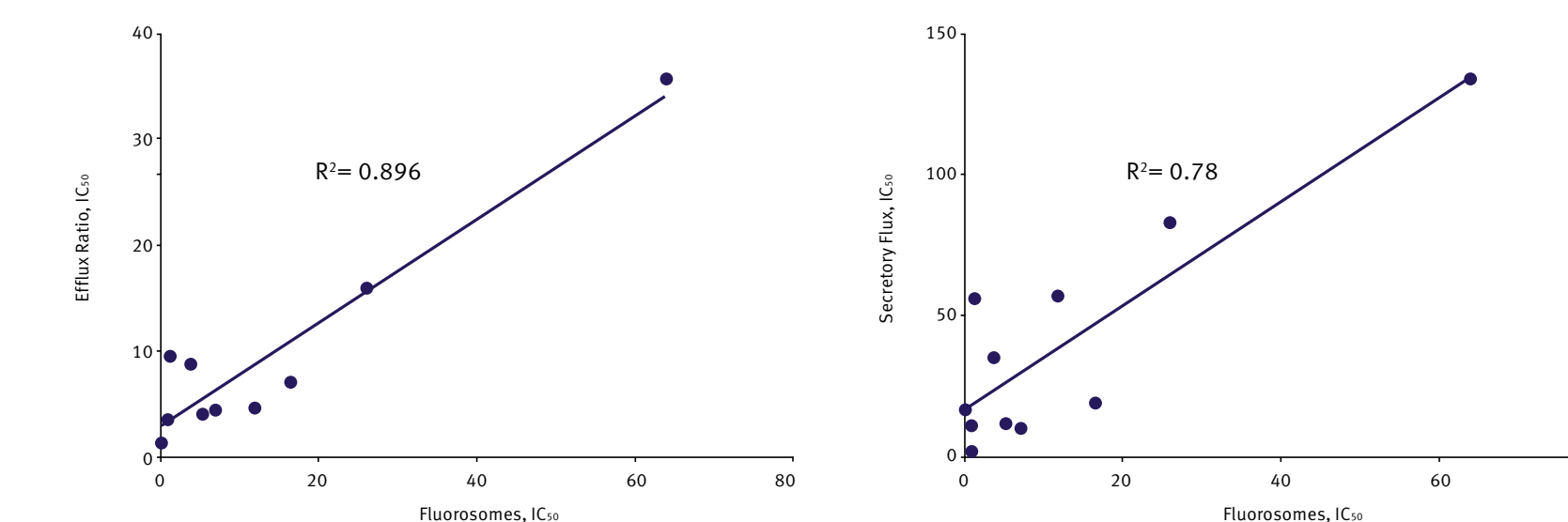


Results and Discussion

Representative Fluorosome-*trans*-pgp IC₅₀ Plots



Correlation of Fluorosome-*trans*-pgp IC₅₀ Data and Cell Monolayer IC₅₀ Data

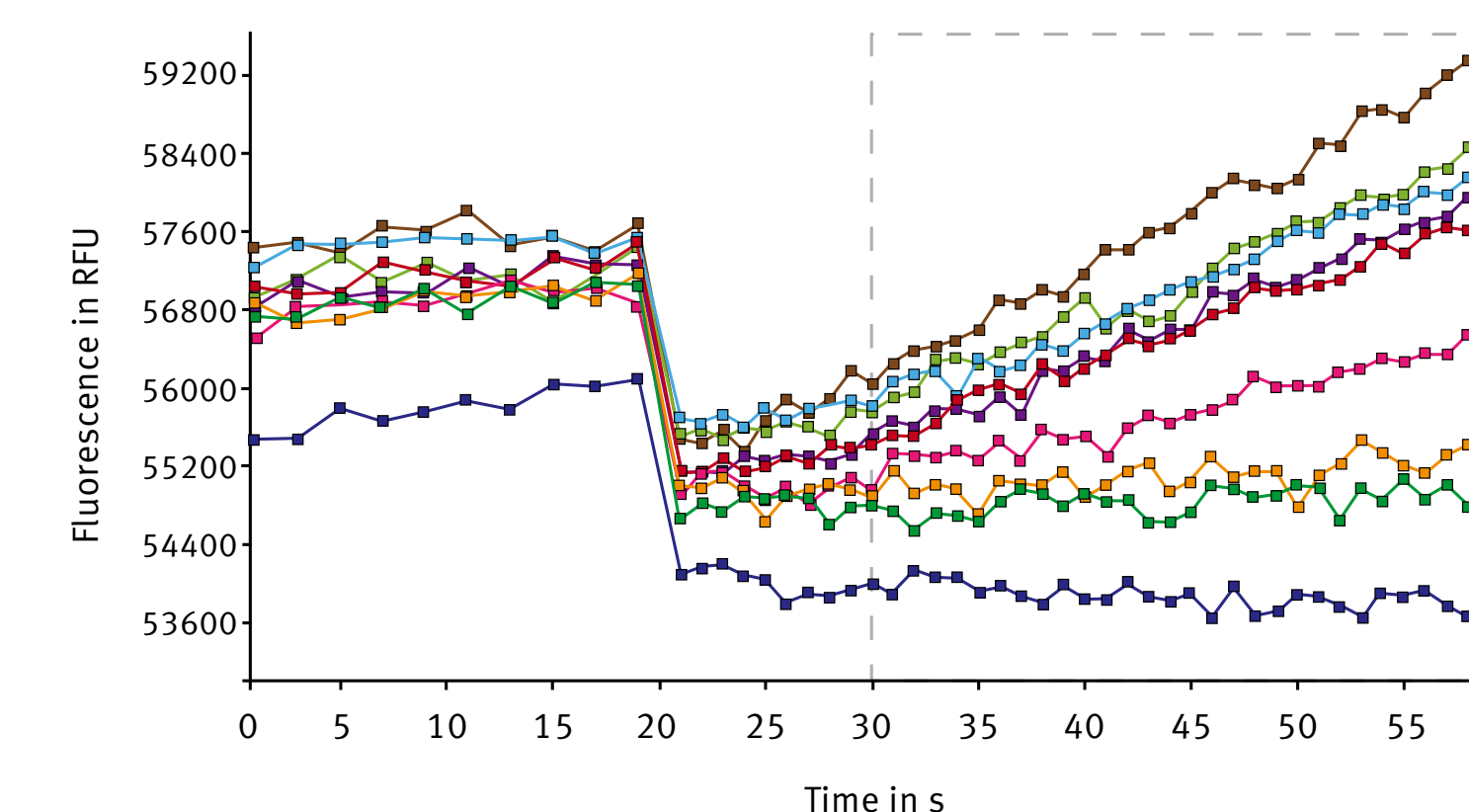


IC₅₀ values are given as µM concentrations. Bidirectional transport inhibition studies were conducted on the same set of compounds as were measured with the Fluorosome-*trans*-pgp assay, examples of which are shown in the left hand panel. Bidirectional transport inhibition studies employed LLC-MDR1 cell monolayers and [³H]digoxin (0.1 µM) as the substrate essentially as described in "Chu et al., 2007, J. Pharmacol. Exp. Ther. 321, 673-683". Cell monolayer IC₅₀ values are reported as Efflux Ratio, i.e. changes in net transport Papp B-A - Papp A-B (upper left panel) and Secretory Flux, i.e. changes in Papp B-A/A-B ratios (upper right panel). Results using the Fluorosome-*trans*-pgp assay are in excellent agreement with those obtained by cell culture methodology.

Materials and Methods

The Fluorosome-*trans*-pgp assay procedure.

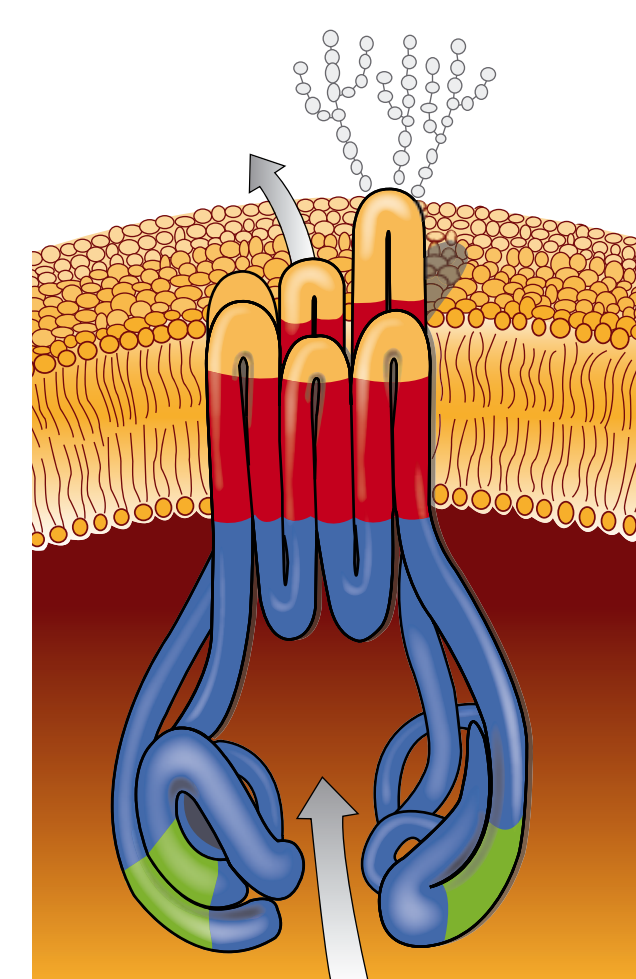
- 98 µl of Fluorosome-*trans*-pgp in buffer containing 3 µM of the substrate S-HR was added to each well of a 96-well half-well plate.
- A 2 µl aliquot of a DMSO solution of test compound was added. Each aliquot contained test compound at one of 7 concentrations to give, after dilution into the well, the desired concentration of compound. Blank, reference wells (no compound) had 2 µl of DMSO added to them.
- The plate was placed in the FLUOstar Omega, and the fluorescence was monitored at 485/520 for 60 seconds as follows:
 - a fluorescence baseline was established during the first 18 seconds;
 - at 18 seconds, 5 µl of a solution of ATP in buffer was injected into each well (final concentration 2 mM), and the fluorescence was monitored for 42 seconds;
 - The slope from 30 to 60 seconds was immediately calculated by BMG LABTECH's MARS data analysis software. (The 10 second interval from 20 to 30 seconds was ignored to obviate any effects arising from mixing turbulence.)



Conclusion

We report a novel assay specific for measuring inhibition of the P-glycoprotein multidrug transporter (Pgp; ABCB1) and an ideal instrument for its use.

- Simple assay procedure: No sterile conditions, non-compound specific, no radiolabel or LC-mass spectroscopy
- Specificity: Fluorosome[®]-*trans*-pgp contains only the P-glycoprotein transporter
- Low Sample Requirement: A 100 µl volume of Fluorosome[®]-*trans*-pgp Reagent per assay requires ~1 nanomole drug
- Speed: 1 minute per inhibition assay - e.g. an 8 point IC₅₀ determination + 2 references takes only 10 minutes
- Popular format: 96 well half-well or 384 well microplates - one well per inhibition assay



Isolation and Purification of Pgp

Purified Pgp (>90%) is isolated from Pgp-overexpressing CH^B30 cells by detergent (CHAPS) extraction followed by affinity chromatography on Con A-Sepharose. Micellar Pgp is reconstituted into lipid bilayers by mixing in solution with CHAPS-solubilized micellar phospholipid, and the detergent is removed by gel exclusion chromatography. The resulting reconstituted Pgp is aliquoted and stored frozen at -70°C.

Manufacture of Fluorosome-*trans*-pgp

Frozen reconstituted Pgp is thawed and mixed in buffer with fluorophore (BSA-fluorescein), then converted into Fluorosome-*trans*-pgp by extrusion. The resulting unilamellar vesicles are separated from unencapsulated fluorophore by gel exclusion chromatography. The fluorosome-*trans*-pgp is then subjected to Quality Control Criteria; size, fluorescence, passive permeability, and ATP-stimulated transport of a control Pgp substrate.