

Cloe Screen Caco-2 Permeability

cyprotexexperts in **ADME**

Background Information



'Studying the permeability of compounds across a Caco-2 cell monolayer is an established *in vitro* model to screen for oral absorption and to evaluate the mechanism of transport. Using LC-MS/MS for the analysis of samples derived from Caco-2 cell studies allows the rapid and accurate determination of drug transport across the Caco-2 cell monolayer.'

¹Wang Z, Hop C.E., Leung K.H. and Pang J. (2000) *J Mass Spectrom* 35 (1): 71-6

- Cloe Screen Caco-2 Permeability assay uses an established method for predicting the *in vivo* absorption of drugs across the gut wall by measuring the rate of transport of a compound across the Caco-2 cell line.
- The Caco-2 cell line is derived from a human colon carcinoma. The cells have characteristics that resemble intestinal epithelial cells such as the formation of a polarised monolayer, well-defined brush border on the apical surface and intercellular junctions.
- Assessing transport in both directions (apical to basolateral (A-B) and basolateral to apical (B-A)) across the cell monolayer enables an efflux ratio to be determined which provides an indicator as to whether a compound undergoes active efflux.
- The P-glycoprotein (P-gp) inhibitor, verapamil, can be included to identify whether active transport is mediated by P-gp.

Prediction of Human Intestinal Absorption

Cloe Screen Caco-2 Permeability data can be used in conjunction with Cloe Screen Turbidimetric Solubility data to predict dose dependent human intestinal absorption. Please refer to our Cloe Predict Human Intestinal Absorption Model section for further details.

Protocol

Test Compound Concentration
10 μ M

Passage Number
40 – 60

Period of Cell Culture
20 days

Number of Replicates
2

Incubation time
2 hours

Temperature
37°C

Compound Requirements
100 μ L of 10 mM DMSO solution

Integrity Marker
Lucifer Yellow

Control Compounds
Atenolol, propranolol and talinolol

Analysis Method
LC-MS/MS quantification

Data Delivery
 P_{app}
Efflux ratio
Experimental Recovery

Cloe Screen Caco-2 assay is performed in a 96-well format providing a cost-effective and highly reproducible method of assessing the permeation potential of test compounds.



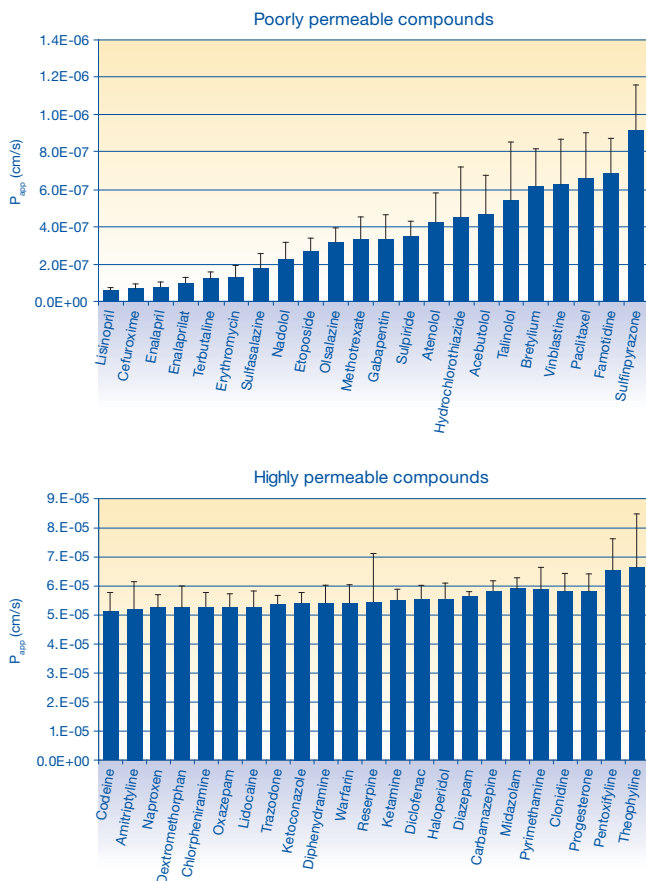
Cloe Screen Caco-2 Permeability

For the validation, a set of compounds were screened through the Cloe Screen Caco-2 Permeability assay over 3 separate experiments. Data generated were reproducible over a range of permeabilities.

The bidirectional assay is able to correctly distinguish between those compounds which are reported to undergo active efflux and those which are not.

Figure 1

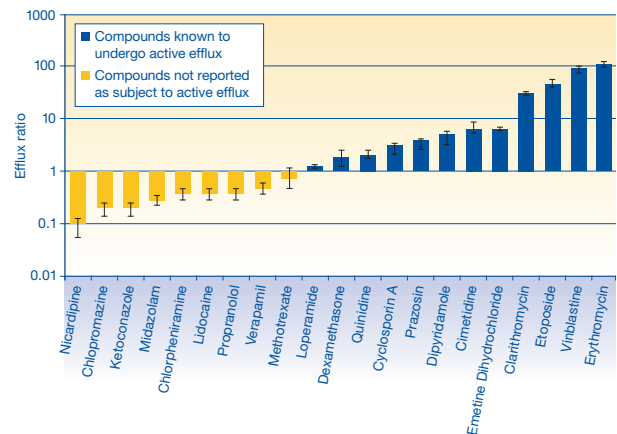
Graph illustrates the consistency of Cloe Screen Caco-2 Permeability data over 3 separate experiments for the apical to basolateral assay.



These data illustrate the high level of reproducibility provided by this assay for a set of compounds with a range of permeabilities.

Figure 2

Graph displays the efflux ratio of a set of 21 compounds generated by the Cloe Screen Caco-2 Permeability assay.



The bi-directional Cloe Screen Caco-2 Permeability assay can identify and quantify levels of active efflux. Screening compounds in both the A to B and B to A direction provides a ratio of B-A/A-B (efflux ratio). When a compound has an efflux ratio of greater than 2, it suggests that the compound may be subject to active efflux.

References

¹ Wang Z *et al.* (2000) *J Mass Spectrom.* 35 (1); 71-6.