

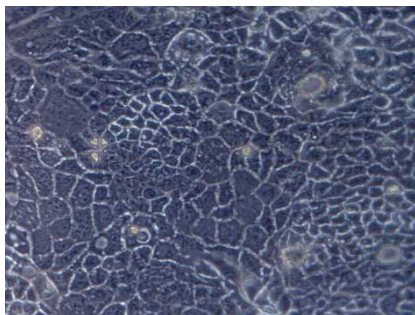
## Permeability Screening Using Caco2 Monolayers

### Purpose

Oral absorption is one of the key success factors of a new compound. Differentiated Caco2 cells exhibit morphological and physiological properties of the human small intestine e.g. barrier function, microvilli, brush-border enzymes, active transport systems and efflux systems. Therefore Caco2 cells provide a well-established *in vitro* model to predict the intestinal absorption *in vivo*.

### Our test model

Caco2 cells are grown on transwell polycarbonate membrane inserts for 21 days. Growth and differentiation is monitored by the measurement of the transepithelial electrical resistance during culture. The test compound is added in triplicate to the donor compartment. Samples are collected from the receiver compartment at specified time points. To assess the influence of P-glycoprotein on the transport process of a compound, the apical-to-basolateral vs. basolateral-to-apical transport is compared. For quality control and for classification of test compounds, reference compounds with a high and low transepithelial flux are included in each assay. Reference compounds can be chosen from the compound list provided by Pharmacelsus.



**Figure 1:** Caco2 monolayer on polycarbonate

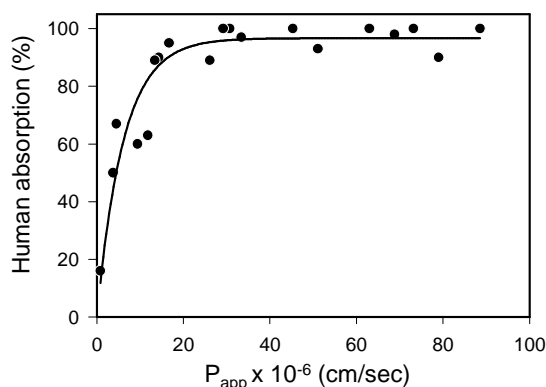
After each assay, the integrity of each cell monolayer is checked by the determination of the permeability coefficient value ( $P_{app}$ ) of Lucifer Yellow.

### Data analysis

Compound concentrations are measured in the receiver and donor compartments by UV-spectroscopy or by LC-MS/MS. These concentration values are used for the calculation of the apparent permeability and the recovery of a compound.

### Model validation

According to the FDA guidelines, validation of our Caco2 permeation assay was performed using reference drugs known as low, moderate or high absorbers. The carrier-mediated transport was determined with known influx and efflux substrates of these transporters.



**Figure 2:** Correlation of permeability in Caco2 cells for a set of 19 reference compounds vs. their human absorption values.

### Assay development and next test step

- For specification of *in vitro* P-glycoprotein interaction, we offer a functional *in vitro* assay using recombinantly expressed Pgp in a membrane fraction.
- The *in vivo* absorption of test compounds can be determined in the rat in our PK catheterized animal model.

Please don't hesitate to contact us for a customized quotation

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