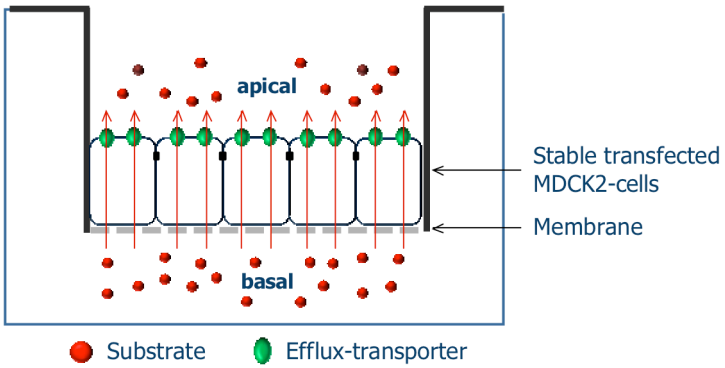
MDCK2 transporter assays

Membrane transporters can be major determinants of the absorption, distribution and elimination of drugs. Moreover, drug interactions with transporters could mediate drug-drug interactions (DDIs). Therefore, the transporter-mediated transport and inhibitory effects on transporter proteins and of drugs is investigated in early stages of drug development. Stable transfected cell lines expressing clinical important transporter proteins are well established tools to characterize the inhibitory effects, the uptake and/or the efflux of drugs.

Trans-membrane assays and calculation of P_{app}-values

Polarized MDCK2-cells expressing an ATP-dependent efflux transporter are seeded to a semi-permeable membrane. A defined concentration of test compound or drug is given in a suitable buffer to the basal donor compartment and incubated at 37°C for up to three hours. Drugs transported to the apical compartment can be quantified by using HPLC, LC-MS/MS or, if radio-labeled substrates are used by liquid scintillation counting.

Test principle trans-membrane assay



Stable transfected MDCK2 cells expressing the transporter protein of interest are incubated with the test compound. Release of test compound is detected using analytical methods like HPLC, LC-MS/MS or others. The apparent permeability coefficient (P_{app}), which describes the active transport of a molecule, is calculated as follows:

$$P_{app} = dQ/dt \times 1/(A \times D_0)$$

where dQ/dt is the cumulative amount of the substance which has been transported over the membrane, A is the area exposed and D_0 is the start concentration of the substrate on the donor side. P_{app} is a rate measured in cm/s.

An active secretion (efflux) is demonstrated if the Efflux ratio (ER) is > 1. The efflux ration is calculated form the P_{app} measured apical-to-basolateral (a-b) and basolateral-to-apical (b-a) according to the following equation:

$$ER = P_{app}\ (b\text{-}a) \,/\, P_{app}\ (a\text{-}b)$$

Recovery/mass balance of the tested compound during the experiment can be calculated as follows:

$$\text{Recovery (\%)} = (C_{end} \times V_{donor} + C_{acceptor}) / (C_0 \times V_{donor}) \times 100\%$$

where C_{end} is the concentration in the donor compartment at the end of the experiment, V_{donor} is the volume of the donor chamber and $C_{acceptor}$ is the total amount of substance transported to the acceptor chamber. An acceptable mass balance is 80 – 120 %.

Available stable transfected MDCK2 cell lines

Efflux transporter:

ABCB1 (MDR1, P-Glycoprotein)

ABCC2 (MRP2, Cmoat)