CONCEPT LIFE SCIENCES

DELIVERING SCIENCE

DATA SHEET

MDCK PERMEABILITY

MDCK (a canine kidney cell line) has been transfected with human genes that encode for efflux transporters, producing modified cell lines that overexpress human transporters. Two such modified cell lines are very useful in assessing test drug efflux potential: 1) MDCK-MDR1 overexpressing human P-gp, and 2) MDCK-BCRP overexpressing human BCRP.

The cell lines are grown to form a monolayer that provides a barrier to test compound between donor and receiver wells, such that most compounds may only traverse by permeability or active transport. This models the barrier between the intestinal lumen and blood supply from hepatic portal vein. **Papp is determined, and compounds that are Pgp substrates are identified.**

CUSTOMER PROVIDES

Compound identifier and molecular formula.

Test: 30µLof 10mM in DMSO or 0.5mg solid.

CELLS

MDCK-MDR1 or MDCK-BCRP cells, cultured 4 days to form a monolayer on 24-well PET inserts. Integrity of monolayer confirmed with TEER > 350Ωcm^2 (before assay) and lucifer yellow < 1 x10^{-6}cm.s^{-1} (after assay).

TEST COMPOUND

Incubation concentration 10µM, n=2 (flexible).

FORMAT

24-well plate, gentle orbital shaking incubator, 2hr at 37°C. Volumes used are 0.4mL apical, and 0.8mL basolateral. Use of shaker minimises the "unstirred water layer effect".

PROTOCOL

Unidirectional transport assay: Test compound is added to apical well (gentle agitation) and transport determined by measuring concentration in basolateral well at 2hr (A → B).

Bidirectional transport assay: At the same time as A → B incubation, a parallel incubation is also performed where test compound is added to a basolateral well and then measured in the apical well (B → A) at 2hr. The efflux ratio is then calculated by comparing the rates of transport in both directions (B → A / A → B) to determine if test compound is an efflux transporter substrate.

Parallel incubations may be performed using Pgp or BCRP specific inhibitors that are co-incubated with test compound to confirm if these transporters are involved. Depending on client preference, alternatively a second plate in the bidirectional assay containing MDCK-WT (wild-type) cells that do not over-express the human transporters may be used. Efflux ratios in the transfected cell line versus MDCK-WT are compared to identify if P-gp or BCRP is involved in the efflux transport.

All donor wells contain lucifer yellow. The final solvent concentration is 0.1% DMSO. After the 2hr incubation, aliquots are removed from all apical and basolateral wells for analysis by LC-MSMS analysis with internal standard against a standard curve. A second set of aliquots are analysed by UV/vis absorbance to against a lucifer yellow standard curve to measure monolayer integrity at the end of the assay.

CONTROLS

Propranolol & quinidine (P-gp), Propranolol & prazosin (BCRP) are used in each assay run. TEER readings are taken prior to the experiment, & lucifer yellow permeability is used in donor wells & measured at the end of the experiment in the receiver wells to show membrane integrity.

QUANTITATION

A standard curve is prepared for each test compound, analysis by LC-MS/MS.

UV/vis absorbance at 492nm is used to measure lucifer yellow in receiver wells against a standard curve.

DATA ANALYSIS AND RESULTS

For each well, response ratio = $\frac{\text{Test Peak Area}}{\text{Standard Peak Area}}$

Response ratio is compared with the standard curve to determine the concentrations in receiver and donor wells. % recovery is calculated to indicate non-specific binding of the test compound.

The apparent permeability Papp is calculated according to:

$$Papp = \frac{V}{A \times Co} \times \frac{dC}{dt}$$

Where V = receiver volume (cm^3).
A = transwell membrane surface area (cm^2).
Co= initial donor well concentration.
dC/dt= rate of concentration change on receiver side ($\mu M.s^{-1}$).

$$\text{Efflux ratio} = \frac{Papp\ (B \text{ to } A)}{Papp\ (A \text{ to } B)}$$

$$\text{Net flux ratio} = \frac{\text{Efflux ratio with MDCK-MDR1cells}}{\text{Efflux ratio with Wild-type cells}}$$