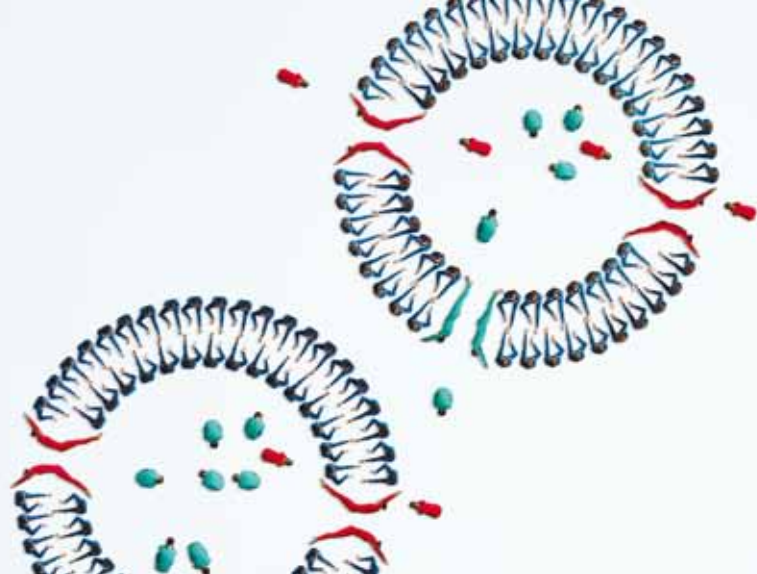


Caco-2 Transporter Knockout Cell Based Assays



CompoZr® Transporter Knockout Cells

To aid in the investigation of specific drug transporters Sigma® has generated single and double transporter knockout (KO) cell lines using CompoZr Zinc Finger Nuclease (ZFN) technology in C2BBE1 cells which are derived from Caco-2 cells. These cells are ideal in that they express multiple transporters, are human derived and grow in a homogenous monolayer that forms tight junctions.

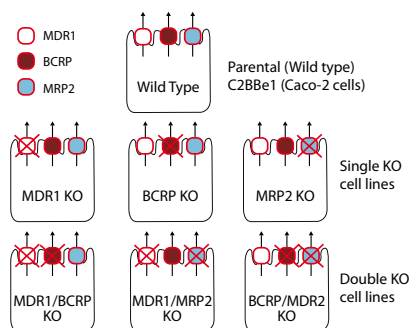


Figure 1. Graphic representation of the transporter knockout(s) in each CompoZr Transporter Knockout Cell Line. The total loss of MDR1, BCRP and/or MRP2 function in each respective cell line enables explicit identification of transporter/substrate interactions.

MDR1 Transporter Knockout Cell Lines

The Efflux Ratios of MDR1 substrates, Digoxin and Erythromycin, were measured in the wild type and single and double MDR1 knockout C2BBE1 cell lines.

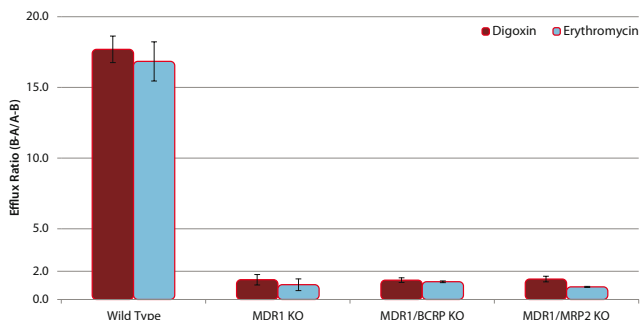


Figure 2. Values are the mean +/- standard deviation, n ≥ 3 assays of triplicates.

BCRP Transporter Knockout Cell Lines

The Efflux Ratios of BCRP substrates, Estrone-3-Sulfate and Nitrofurantoin, were measured in the wild type and single and double BCRP knockout C2BBE1 cell lines.

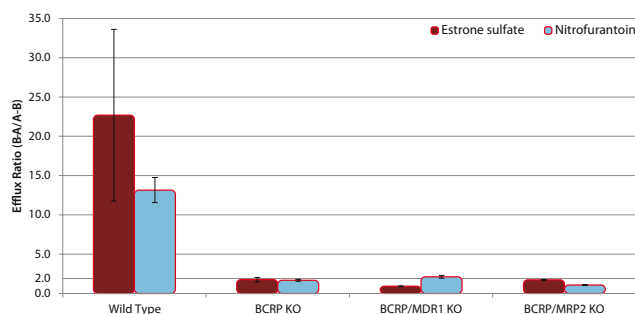


Figure 3. Values are the mean +/- standard deviation, n ≥ 3 assays of triplicates.

MRP2 Transporter Knockout Cell Lines

The Efflux Ratio of the MRP2 substrate, CDCF, was measured in the wild type and single and double MRP2 knockout C2BBE1 cell lines.

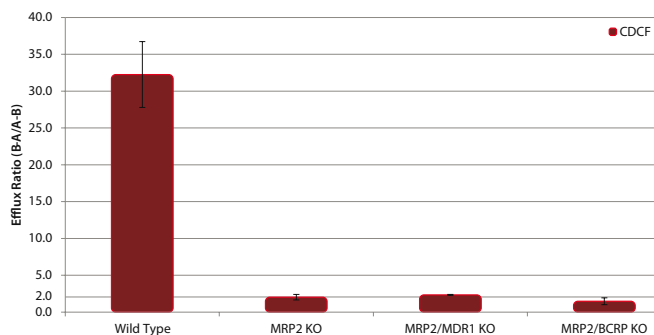


Figure 4. Values are the mean +/- standard deviation, n ≥ 3 assays of triplicates.

Case Studies with Transporter Knockouts

Analysis of Cimetidine with the MDR1/BCRP Double Knockout Cell Line

Cimetidine is an example of a crossover substrate that has an efflux ratio > 2 in each of the Single Transporter Knockout Cell Lines. When analyzed with the Double Transporter Knockout Cell Lines the efflux ratio for the MDR1/BCRP double knockout cell line is < 2. This total loss of active transport indicates cimetidine is a substrate for both MDR1 and BCRP.

The data indicate further research with both transporters would be necessary for cimetidine.

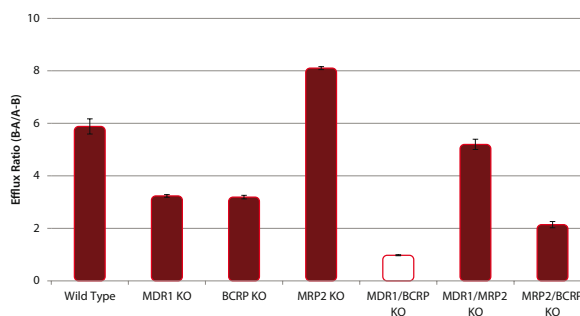


Figure 5. Efflux Ratios for Cimetidine
Values are the mean +/- standard deviation, n ≥ 3 assays of triplicates.

Combination of Transporter Knockout Cell Lines and Chemical Inhibitors

To determine transporter/substrate interactions with crossover substrates or with substrates that are actively effluxed by transporters other than MDR1, BCRP and MRP2 chemical inhibitors may be utilized. Transport of the substrate sulfasalazine is not prevented in any of the CompoZr Transporter Knockout Cell Lines. The combination of transporter knockouts and the chemical inhibitors MK571 (MRP family) and Ko143 (BCRP family) were used to further elucidate the active transporters for this compound.

Total loss of sulfasalazine transport is observed in all BCRP knockout cell lines in the presence of MK571 or in the MRP2 and MDR1/MRP2 knockout cell lines in the presence of both inhibitors. These data suggest sulfasalazine is a substrate for BCRP but is also effluxed by an unidentified transporter in C2BBE1 cells such as MRP3, 4 or 6.

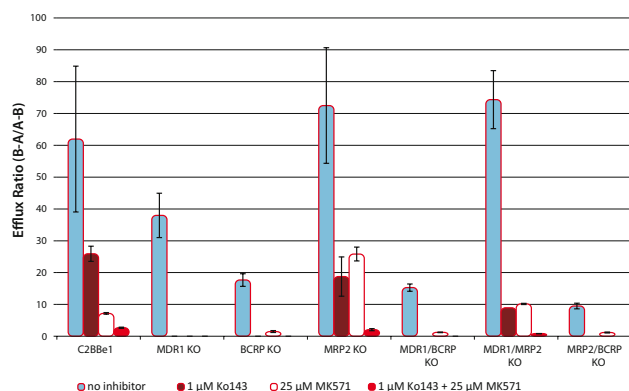


Figure 8. Transport of Sulfasalazine
Values are the mean +/- standard deviation, n ≥ 3 assays of triplicates.

Product Availability

- **Assay Ready Plates** – 24 or 96-well Transwell Assay Plates that contain a monolayer of CompoZr Transporter Knockout Cells ready for efflux ratio analysis upon receipt
- **Licensing** – For profit institutions may license any of the Transporter Knockout Cell Lines
- **Service** – Sigma has licensed the CompoZr Transporter Knockout Cells with select CROs to enable efflux ratio analysis to be conducted by a third party

To learn more about Transporter Knockout Cell Lines, visit sigma.com/transporterko

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