Kidney SLC Transporter Toxicity Models: RPTEC/TERT1-OAT1 and OCT2
Kidney transporter over-expressing cell lines compared with parental RPTEC/TERT1. OAT1 and OCT2 clones display the same renal epithelial growth pattern as parental RPTEC/TERT1 cells.
Molecular characterization of the RPTEC SLC transporter cells. **Left** Immunoblot demonstrates OAT1 protein expression levels in RPTEC/TERT1 parental and OAT1 cells. **Right** Immunoblot demonstrates OCT2 protein expression levels RPTEC/TERT1 parental and OCT2 cells.
OAT1, and OCT2 presence detected in RT-PCR early and late passage

Molecular characterization of the RPTEC SLC transporter cells. **Top** RT-PCR demonstrates the presence of OAT1 mRNA in RPTEC/TERT1 OAT1 cells. **Bottom** RT-PCR demonstrates the presence of OCT2 mRNA in RPTEC/TERT1 OCT2 cells.
Surface markers and method of detection: OAT1 and OCT2

Molecular characterization of the RPTEC SLC transporter cells. Left) IF/ICC demonstrates OAT1 expression and localization. Right) IF/ICC demonstrates OCT2 expression and localization.
Surface markers and method of detection: OAT1 and OCT2

Kidney transporter over-expressing cell lines compared with parental RPTEC/TERT1. RPTEC/TERT1 SLC transporter cells were subjected to immunostaining and dome formation assay. OAT1 and OCT2 clones display the same renal epithelial growth pattern as parental RPTEC/TERT1 cells. The renal epithelial markers CD13 and E-cadherin are expressed in both parental RPTEC/TERT1 cells and in the OAT1 and OCT2 lines.
Figure 3. Kidney transporter over-expressing cell lines compared with parental RPTEC/TERT1. RPTEC/TERT1 SLC transporter cells were subjected to dome formation assay. OAT1 and OCT2 clones display the same renal epithelial growth pattern as parental RPTEC/TERT1 cells.
Evidence of transporter functionality - OAT1 transporter assay, 5-CF (6-CF)

Use 5-CF as uptake substrate

Use 6-CF as uptake substrate

Drug Kinetic Profiles of RPTEC/TERT1 OAT1 Transporter Cells. Solute uptake activity of RPTEC/TERT1 OAT1 cells was assessed using 5-CF or 6-CF as substrate. As expected, uptake increases with increasing 5-CF or 6-CF concentration in OAT1-expressing cells but not in parental RPTEC/TERT1 cells (n=3), indicating that the observed transport is due to OAT1 expression.
Evidence of transporter functionality - OAT1 transporter assay, 5-CF (6-CF)-uptake inhibition

Transport inhibition kinetics of RPTEC/TERT1 OAT1-expressing cell lines. OAT1-expressing cells were exposed to increasing concentrations of the known OAT1 inhibitors probenecid and novobiocin while 5-CF or 6-CF concentration and uptake time were held constant at 100 µM and 20 minutes, respectively. The resulting inhibition curves indicate that OAT1 has physiologically relevant transport activity when overexpressed in RPTEC/TERT1 cells (n=3).
Evidence of transporter functionality - OCT2 transporter assay, Asp+(EAM-1)

Drug Kinetic Profiles of RPTEC/TERT1 OCT2 Transporter Cells. Solute uptake activity of RPTEC/TERT1 OCT2 cells was assessed using ASP+ or EAM-1 as substrate. As expected, uptake increases with increasing amounts of ASP+ and EAM-1 in OCT2-expressing cells but not in parental RPTEC/TERT1 cells (n=3), indicating that the observed solute transport is due to OCT2 expression.
Evidence of transporter functionality- OCT2 transporter assay, Asp+(EAM-1)-uptake inhibition

Use ASP+ as uptake substrate

Use EAM-1 as uptake substrate

Transport inhibition kinetics of RPTEC/TERT1 OCT2-expressing cell lines. OCT2 expressing cells were exposed to increasing concentrations of the known OCT2 inhibitors cimetidine and quinitin while ASP+ concentration and uptake time were held constant at 100 µM and 20 minutes, respectively. The resulting inhibition curves indicate that OCT2 has physiologically relevant transport activity when overexpressed in RPTEC/TERT1 cells (n=3).