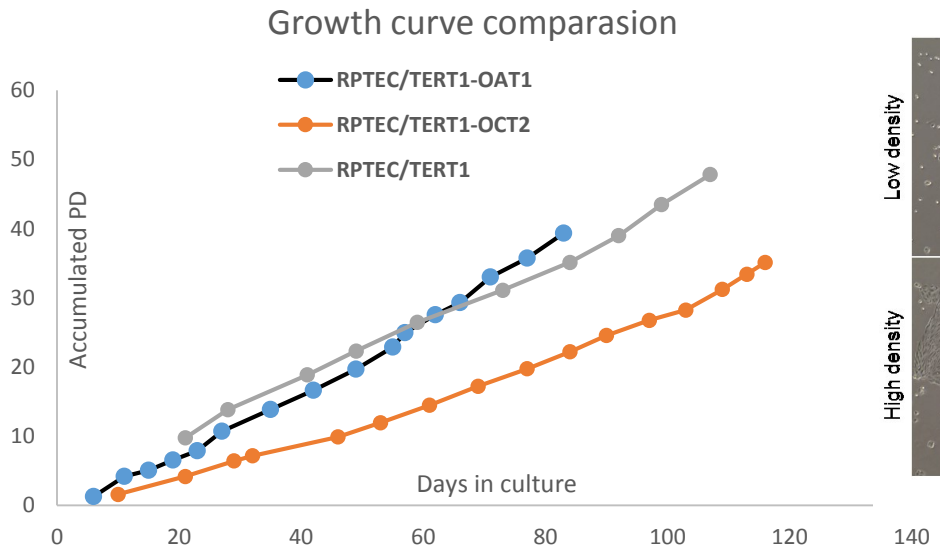
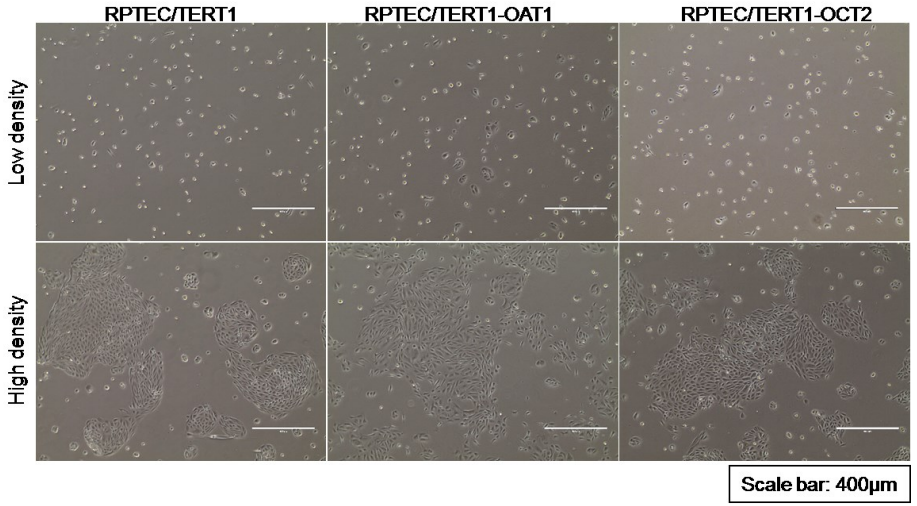
Kidney SLC Transporter Toxicity Models: RPTEC/TERT1-OAT1 and OCT2

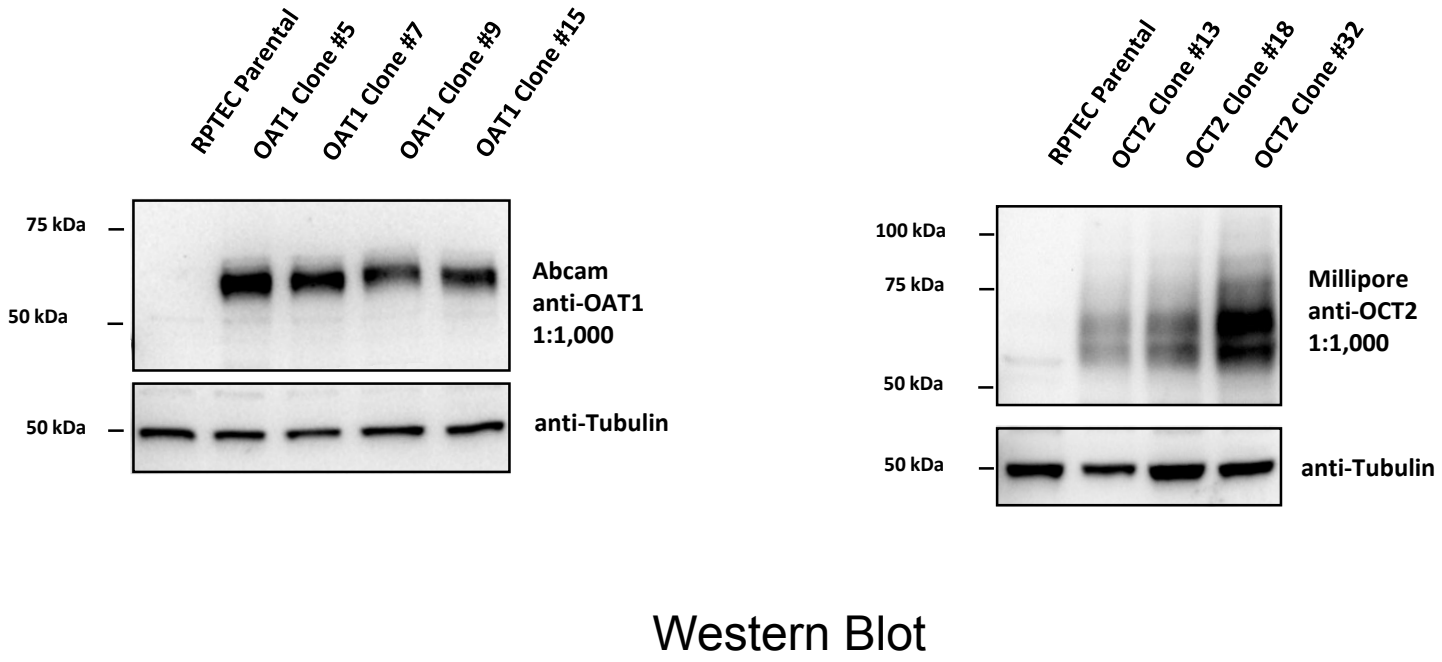
Growth Curve and Morphology





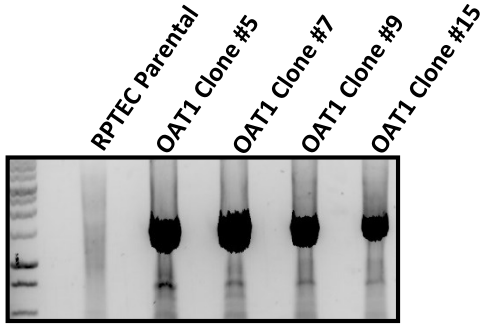
Kidney transporter over-expressing cell lines compared with parental RPTEC/TERT1. OAT1 and OCT2 clones display the same renal epithelial growth pattern as parental RPTEC/TERT1 cells.

OAT1, OCT2 western blot, presence of OAT1, OCT2 when compared to parental line

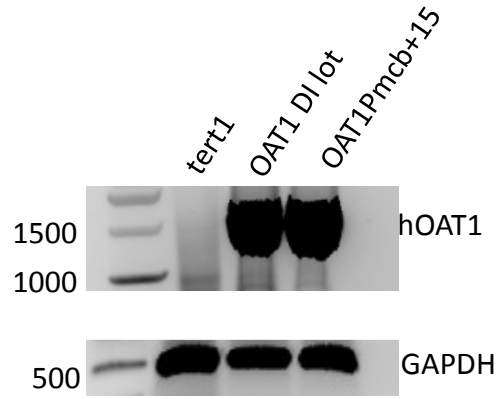


Molecular characterization of the RPTEC SLC transporter cells. Left) Immunoblot demonstrates OAT1 protein expression levels in RPTEC/TERT1 parental and OAT1 cells. **Right)** Immunoblot demonstrates OCT2 protein expression levels RPTEC/TERT1 parental and OCT2 cells.

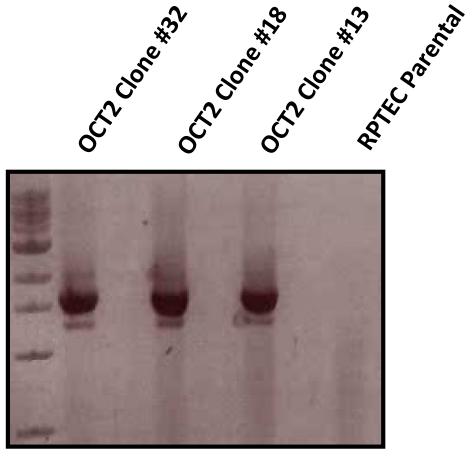
OAT1, and OCT2 presence detected in RT-PCR early and late passage



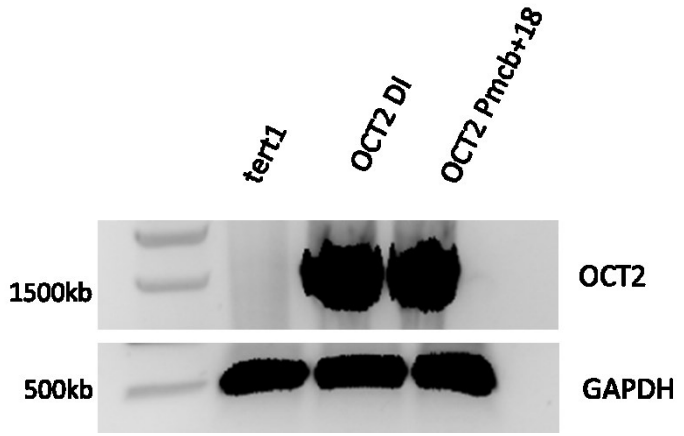
RT-PCR



MCB, DI and MCB+15 passages

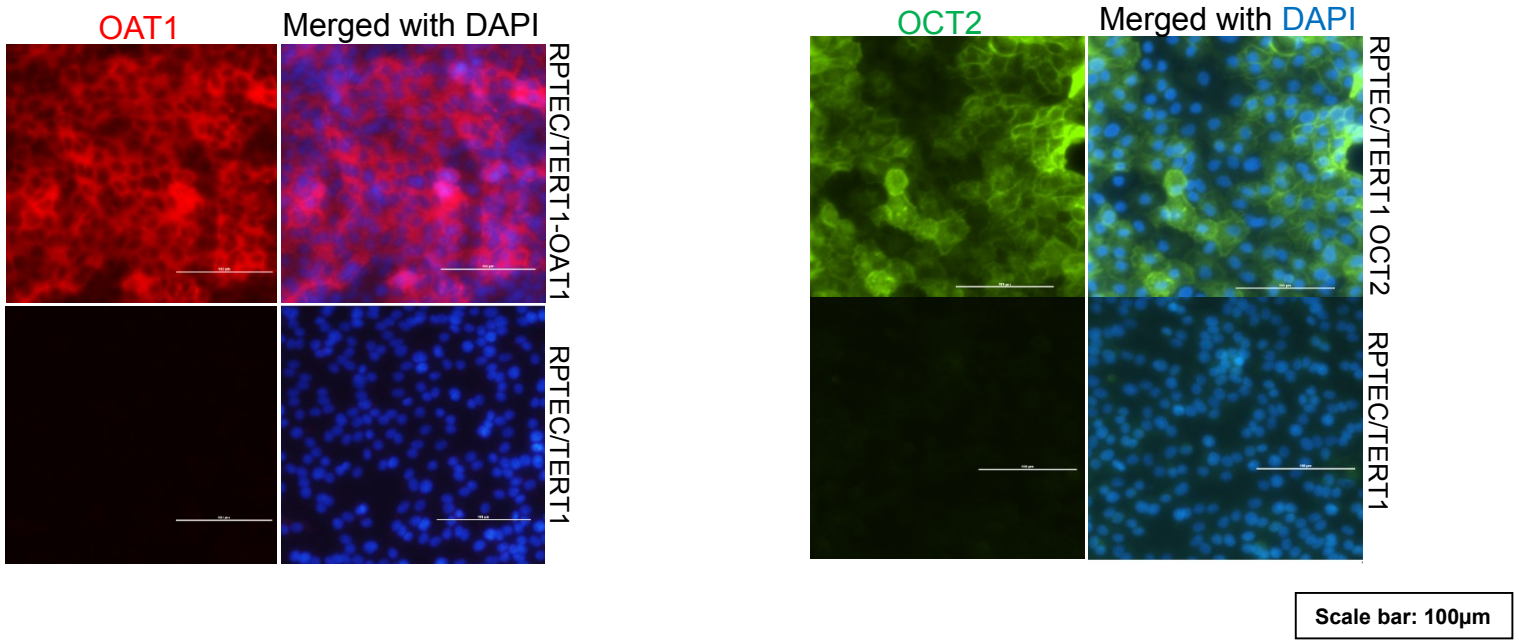


RT-PCR



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

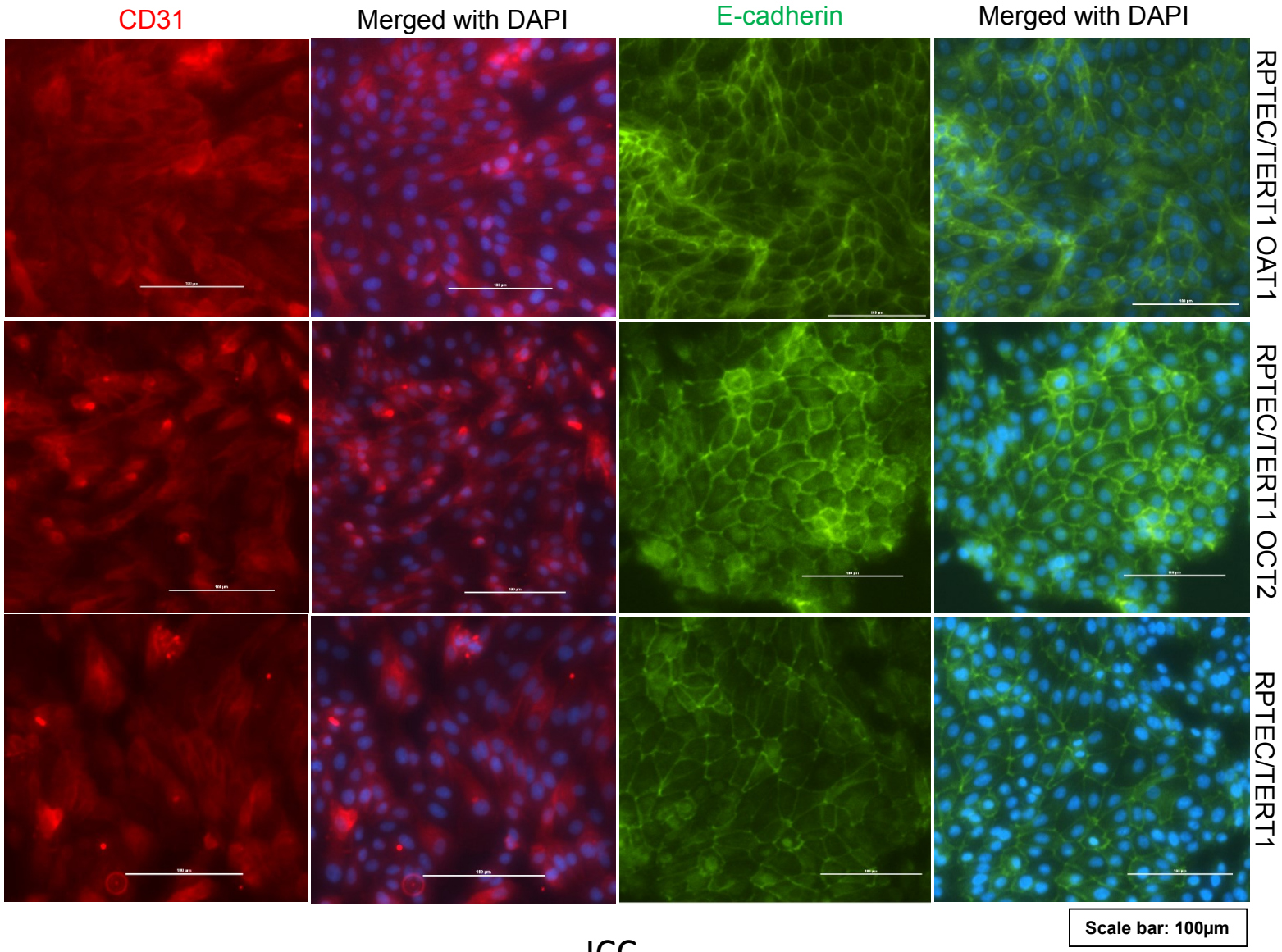


ICC

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.



ICC

Dome formation

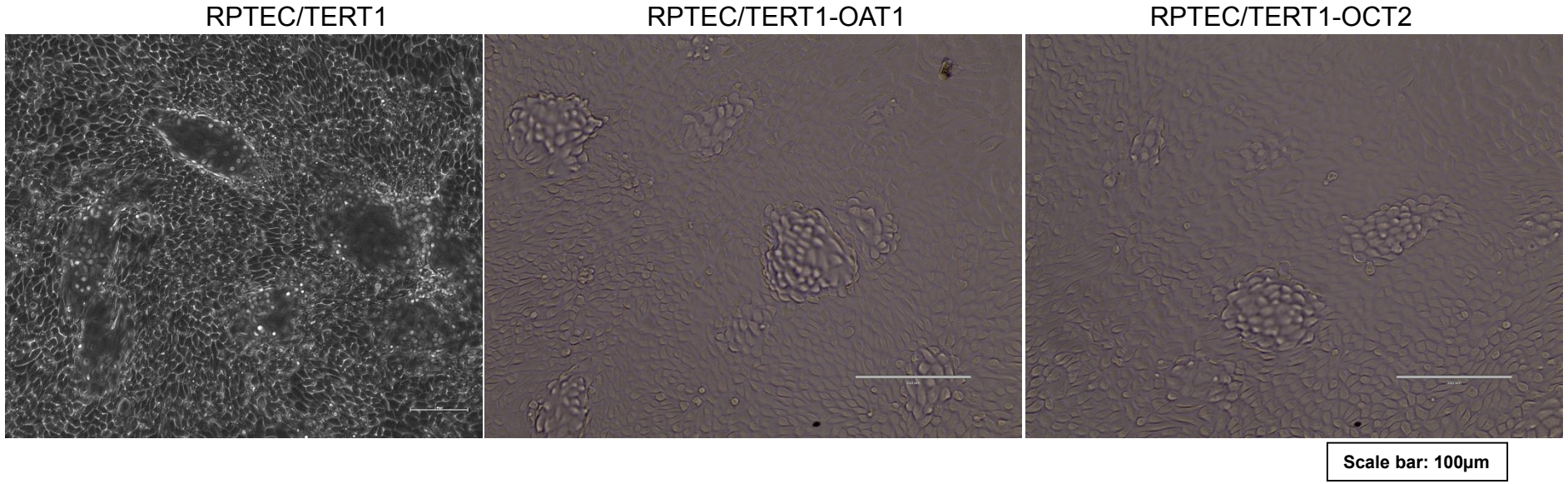
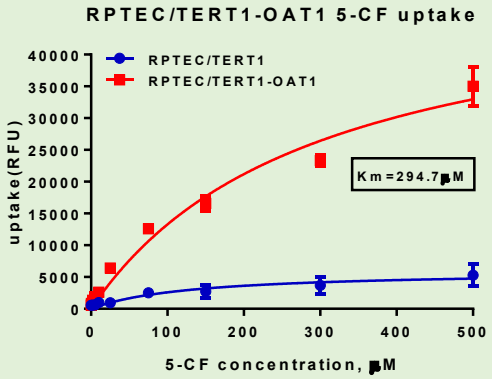


Figure 3. Kidney transporter over-expressing cell lines compared with parental RPTEC/TERT1. RPTEC/ TERT1 SLC transporter cells were subjected to dome formation assay. OAT1 and OCT2 clones display the same renal epithelial growth pattern as parental RPTEC/TERT1 cells.

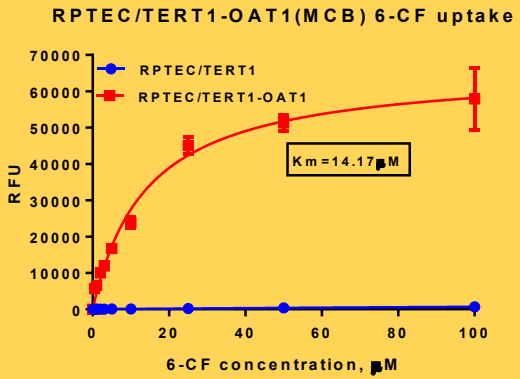
Evidence of transporter functionality- OAT1 transporter assay, 5-CF (6-CF)

Use 5-CF as uptake substrate



5-CF con(μ	0	0.5	2	5	10	25	75	150	300	500
uptake rati	1.061636	1.512075	2.323561	3.1003	2.633725	6.718272	5.00061	6.100167	6.350512	6.641002

Use 6-CF as uptake substrate

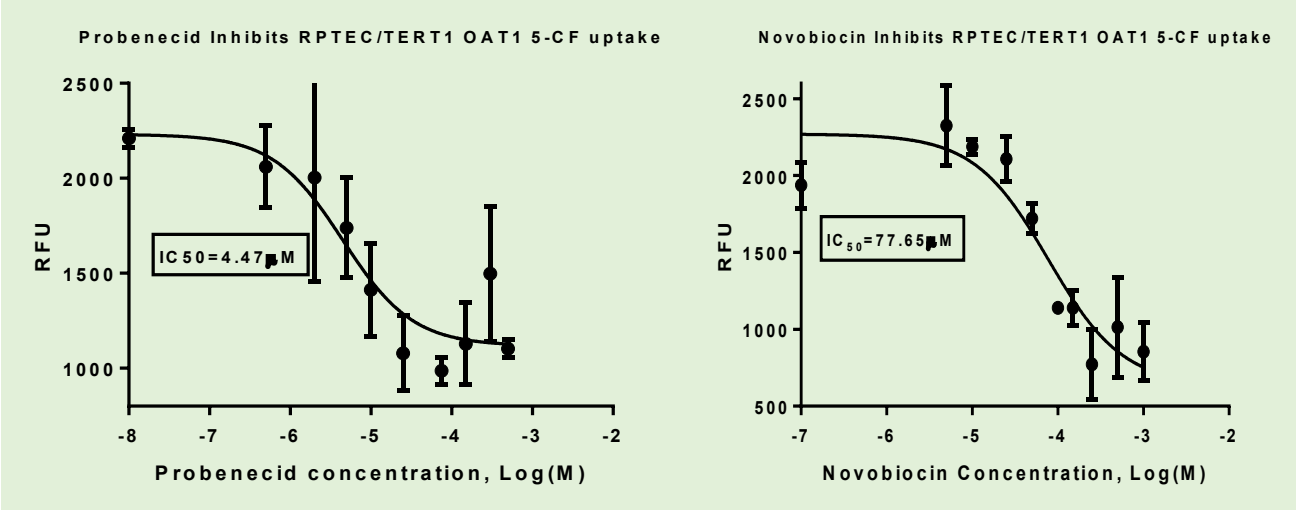


6-CF conc.	0μM	0.5μM	1.0μM	2.0μM	3.0μM	5.0μM	10.0μM	25.0μM	50.0μM	100.0μM
uptake rati	1.160812	169.6708	226.9443	239.1342	260.7467	274.1321	287.706	198.1971	146.0713	84.16204

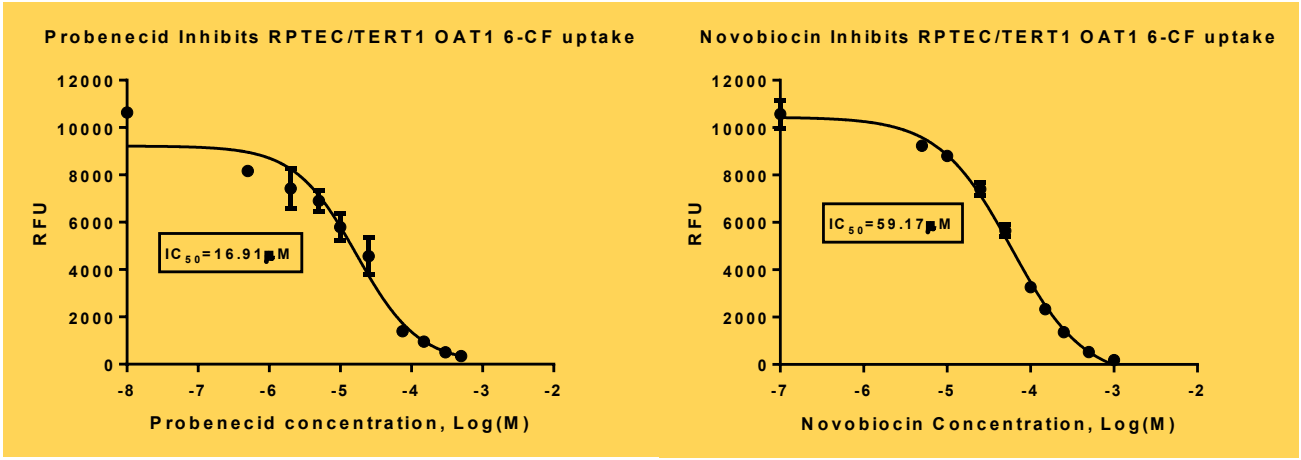
Drug Kinetic Profiles of RPTEC/TERT1 OAT1 Transporter Cells. Solute uptake activity of RPTEC/TERT1 OAT1 cells was assessed using 5-CF or 6-CF as substrate. As expected, uptake increases with increasing 5-CF or 6-CF concentration in OAT1-expressing cells but not in parental RPTEC/TERT1 cells (n=3), indicating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

Evidence of transporter functionality- OAT1 transporter assay, 5-CF (6-CF)-uptake inhibition

Use 5-CF as uptake substrate



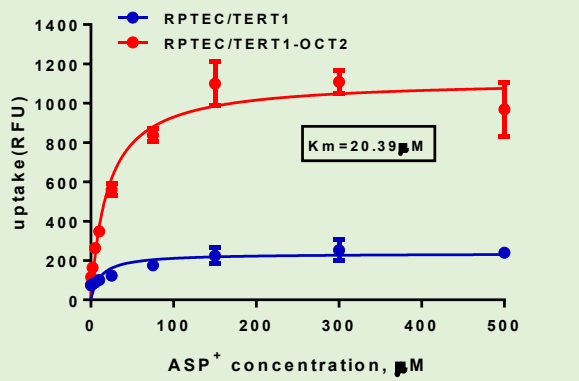
Use 6-CF as uptake substrate



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).

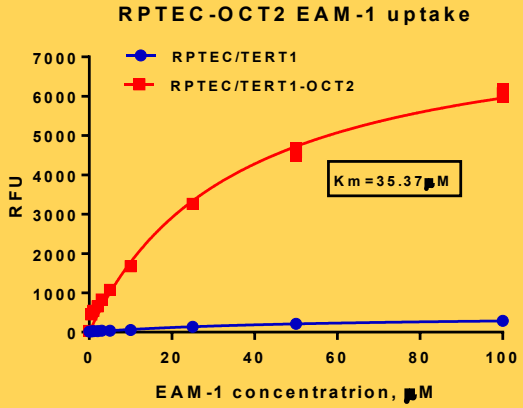
Evidence of transporter functionality- OCT2 transporter assay, Asp+(EAM-1)

Use ASP+ as uptake substrate



Asp con	0μM	0.5μM	2μM	5μM	10μM	25μM	75μM	150μM	300μM	500μM
ratio	1.017543	1.523934	2.033221	2.989123	3.455444	4.540586	4.776906	4.883032	4.38785	4.050386

Use EAM-1 as uptake substrate

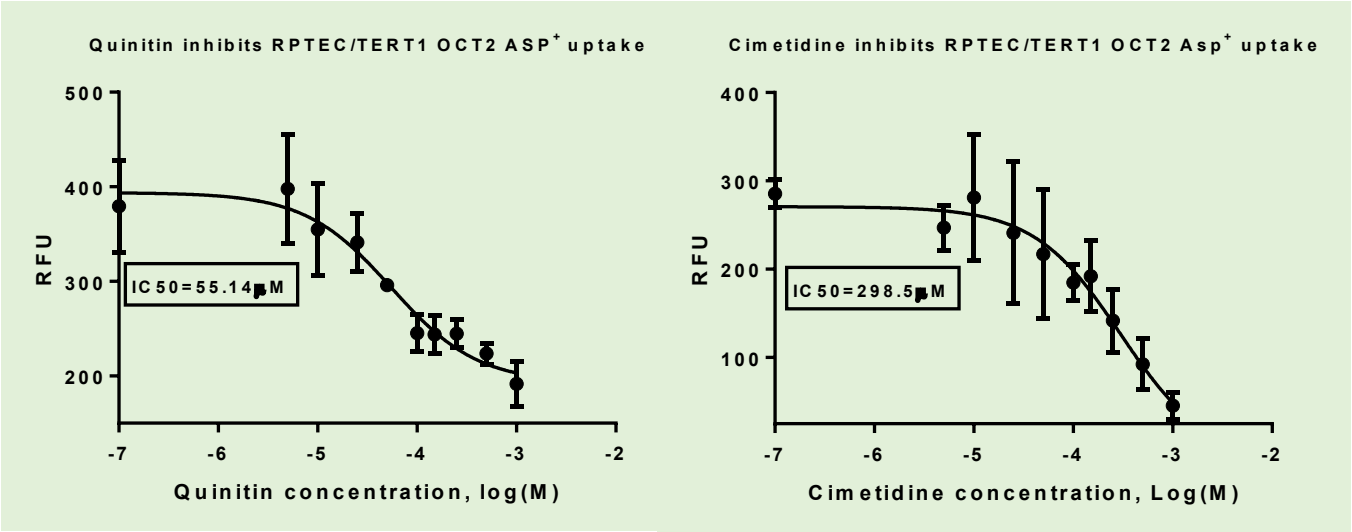


EAM-1 cor	0μM	0.5μM	1μM	2μM	3μM	5μM	10μM	25μM	50μM	100μM
uptake rat	2.267334	21.56116	23.0057	22.65206	22.73024	28.13118	29.92067	23.72269	21.58114	21.21723

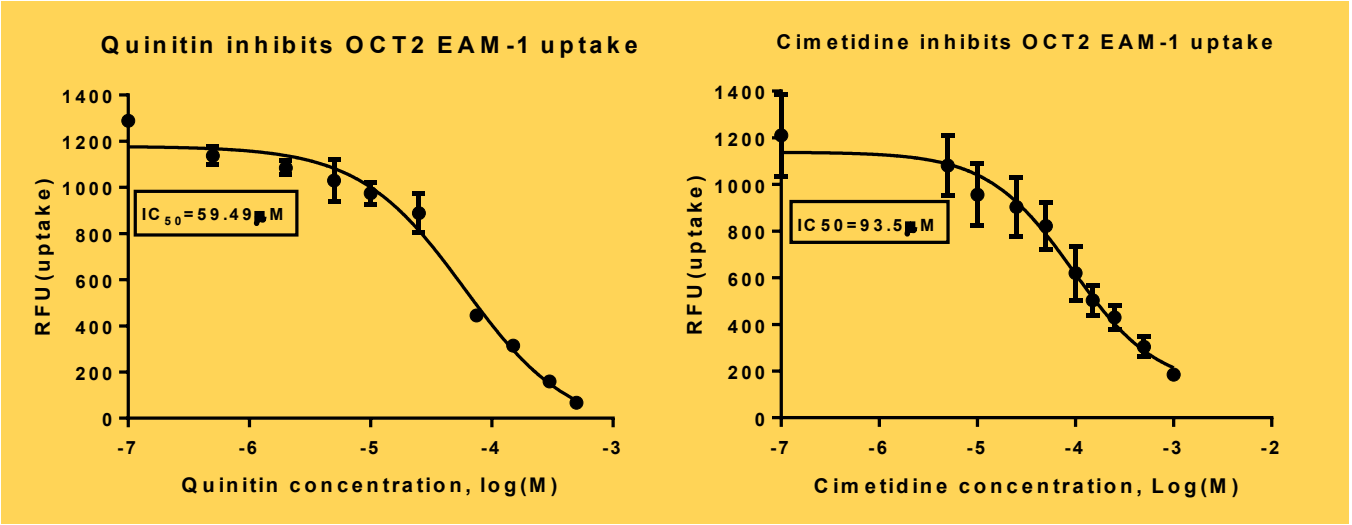
Drug Kinetic Profiles of RPTEC/TERT1 OCT2 Transporter Cells. Solute uptake activity of RPTEC/TERT1 OCT2 cells was assessed using ASP+ or EAM-1 as substrate. As expected, uptake increases with increasing amounts of ASP+ and EAM-1 in OCT2-expressing cells but not in parental RPTEC/TERT1 cells (n=3), indicating 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).