Protocol for RPTEC/TERT1-OAT1 (ATCC CRL-4031-OAT1) Uptake of 5-Carboxyfluorescein (5-CF) or 6-Carboxyfluorescein (6-CF)

There is an abundance of OAT1 transporter in kidney tissue. However, primary cells lose OAT1 expression after just a few days in culture. To facilitate hOAT1 *in vitro* toxicity studies we have generated OAT1 RPTEC/TERT1 (ATCC® CRL-4031-OAT1™) cell line by stably expressing hOAT1 into the RPTEC/TERT1 (ATCC® CRL-4031™) cell line.

Functionality of the OAT1 RPTEC/TERT1 can be demonstrated by 5-Carboxyfluorescein (5-CF) or 6-Carboxyfluorescein (6-CF). 5-CF or 6-CF is a fluorescent in the GREEN channel and is readably been taken up by the OAT1 transporter, after uptake, the cells can be visualized under a fluorescent microscope or lysed and read on a fluorescent plate reader.

Materials:

Material	Company	Catalogue Number
RPTEC/TERT1-OAT1	ATCC	CRL-4031-OAT1
RPTEC/TERT1	ATCC	CRL-4031
HBSS	Corning	21-023-CV
5-CF	Sigma	86826
6-CF	Thermo Fisher	C1360
Black 96-well plates	Corning	354649
M-Per lysis buffer	Thermo Fisher	78501
RPTEC media		
Basal media, DMEM:F12	ATCC	30-2006
Growth kit	ATCC	ACS-4007

Protocol:

A. Seeding cells

1. Make complete RPTEC media by adding 5 mL of component A and 8mL of component B to the basal DMEM:F12 media, mix well.
2. Thaw both RPTEC/TERT1 and RPTEC/TERT1-OAT1 cells and seed at 1.5 x 10^4 to 2.0 x 10^4 cells/cm^2 in T75cm tissue culture flasks in RPTEC media (for RPTEC/TERT1-OAT1, need to add 0.3 µg/mL puromycin for long term culturing).

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Passage cells at least one time prior to seeding cells in 96-well plates for the uptake assay.

3. Trypsinize both RPTEC/TERT1 and RPTEC/TERT1-OAT1 cells and re-suspend each cell line at a concentration of 5 x10^5 cells/mL in RPTEC media without antibiotic.
4. Plate 200 µL of cells into each well of a 96-well collagen I coated plate ensuring that both cells have a minimum of three replicates each.
5. Incubate the cells for 24–36 hours at 37ºC and 5% CO_2 incubator.
6. Making 100 mM 5-CF or 6-CF stock in DMSO.

B. 5-CF/6-CF uptake assay

1. Make sure the cells are more than 90% confluent before you start the assay.
2. Wash the cells two times with 200 µL warm (37ºC) HBSS.
3. Incubate the 2nd wash at 37ºC and 5% CO_2 for 10 minutes; get rid of the remaining liquid using pipet to the last drop.
4. Add 100 µL of 100 µM of 5-CF or 3 µM of 6-CF in warm HBSS and incubate at 37ºC and 5% CO_2 for 20 minutes.
5. Get rid of the 5-CF or 6-CF by using pipet tips to the last drop
6. Wash 4 times with cold (4ºC) HBSS.
7. (Optional) The last wash can remain on the cells for up to 10 minutes to visualize the cells in the Green channel.
8. Remove the wash using pipet tips to the last drop and add 100 µL of M-Per lysis buffer.
9. Incubate for 10 minutes at room temperature protected from light.
10. Read on a fluorescent plate reader at 490ex/510-580$_{em}$.
11. Calculate the fluoresce intensity ratio of 5-CF or 6-CF over parental.

$$\textbf{Uptake ratio} = \frac{\textbf{RFU of (RPTEC/TERT1-OAT1-Blank)}}{\textbf{RFU of (RPTEC/TERT1-Blank)}}$$

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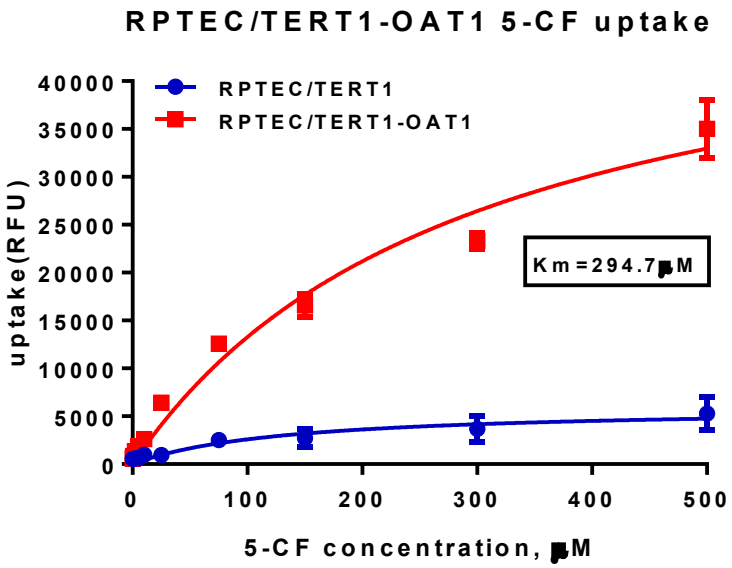
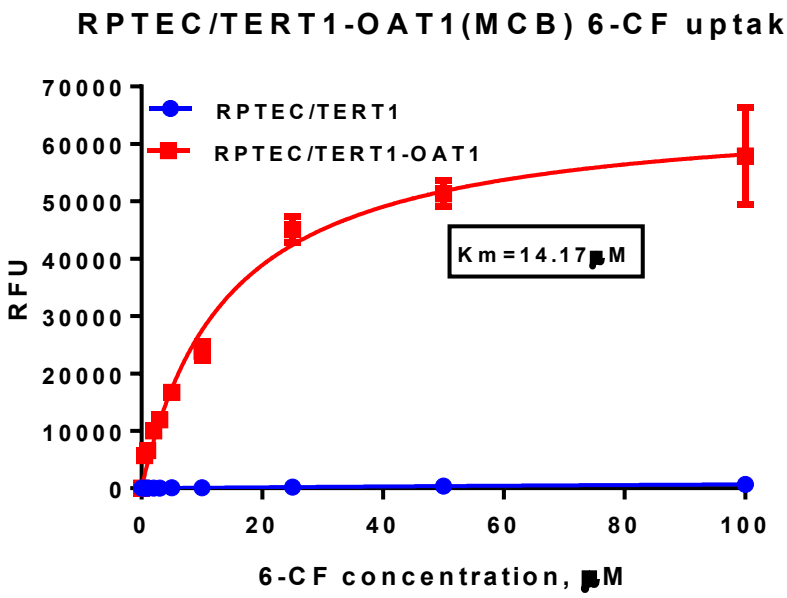


5-CF concentration	0μM	0.5μM	2μM	5μM	10μM	25μM	75μM	150μM	300μM	500μM
uptake ratio	1.061636	1.512075	2.323561	3.1003	2.633725	6.718272	5.00061	6.100167	6.350512	6.641002

Fig.1. RPTEC/TERT1-OAT1 5-CF uptake assays: cells were seeded on 96 well plate, 24 hours later, different concentration of 5-CF were added in the HBSS buffer and incubate at 37°C for 20 minutes; uptake was stopped by washing the wells with ice-cold HBSS, after 4 wash of the HBSS, a 100 µL of lysate buffer was added to each well, florescence intensity is measured using the Promega GloMax®-Multi Detection System.



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

Fig.2. RPTEC/TERT1-OAT1 6-CF uptake assays: cells were seeded on 96 well plate, 24 hours later, different concentration of 6-CF were added in the HBSS buffer and incubate at 37°C for 20 minutes; uptake was stopped by washing the wells with ice-cold HBSS, after 4 wash of the HBSS, a 100 µL of lysate buffer was added to each well, fluorescence intensity is measured using the Promega GloMax®-Multi Detection System