Protocol for OAT1 HEK 293T/17 Uptake of 5-Carboxyfluorescein (5-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 HEK 293T/17 (ATCC® CRL-11268G-1™) cell line by stably expressing hOAT1 into the HEK 293T/17 (ATCC® CRL-11268™) cell line.

Functionality of the OAT1 HEK 293T/17 was demonstrated by uptake of 5-Carboxyfluorescein (5-CF). 5-CF is a fluorescent in the FITC channel and is readably 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
OAT1 HEK 293T/17	ATCC	CRL-11268G-1
HEK 293T/17	ATCC	CRL-11268
HBSS	Corning	21-023-CV
5-CF	Sigma	86826
Black 96-well plates	Corning	354640
M-Per lysis buffer	Thermo Fisher	78501
DMEM	ATCC	30-2002
FBS	ATCC	30-2020

Protocol:

A. Seeding cells

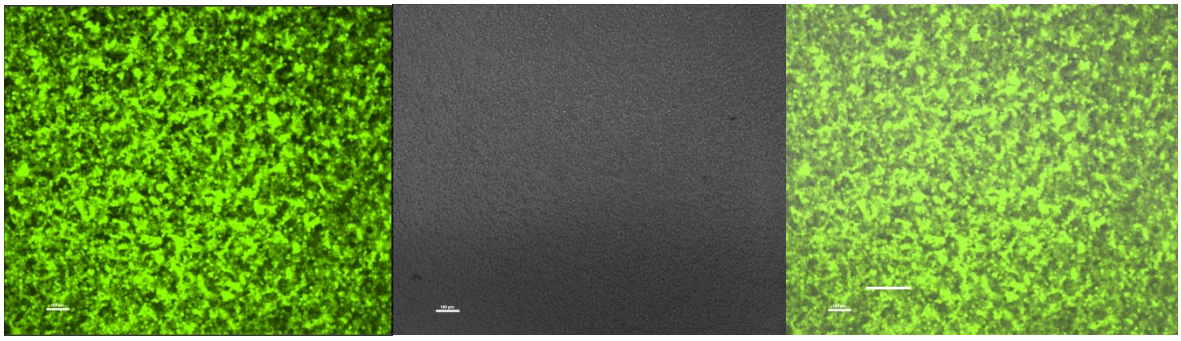
1. Thaw or split both HEK 293T/17 and OAT1 HEK 293T/17.
2. Resuspend the each cell line at a concentration of $5x10^5$ cells/mL in DMEM with 10% FBS.
3. Plate 200µL of cells into each well of a 96-well poly-D-lysine coated plate ensuring that both cells have a minimum of three replicates each.
4. Incubate the cells for 18 – 28 hours at 37°C and 5% CO_2 incubator.

B. 5-CF assay

American Type Culture Collection
P.O. Box 1549
Manassas, VA 20108 USA
http://www.atcc.org

800-683-6597 or 703-365-2700
Fax: 703-365-2750
E-mail: tech@atcc.org
Or contact your local distributor.

1. Make sure the cells are more than 90% confluent before you start the assay.
2. Wash the cells three times with 200 µL warm (37ºC) HBSS[1].
3. Incubate the third wash at 37ºC and 5% CO_2 for 10 minutes.
4. Add 200 µL of 150 µM of 5-CF in warm HBSS and incubate at 37ºC and 5% CO_2 for 20 minutes[2].
5. Wash three times with cold (4ºC) HBSS.
6. (Optional) The last wash can remain on the cells for up to 10 minutes to visualize the cells in the FITC channel.
7. Remove the wash and add 100 µL of M-Per lysis buffer.
8. Incubate for 5 minutes at room temperature protected from light.
9. Read on a fluorescent plate reader at 490_{ex} and $510\text{-}580_{em}$.
10. Calculate the signal of OAT over parental to determine the relative ratio. Or the parental can be used to subtract background.



5-CF **Phase** **Merged**

Figure1. Uptake of 5-CF by OAT1 HEK 293T/17 (ATCC® CRL-11268G-1™)
OAT1 HEK 293T/17 cells after taking up 5-CF visualized using a Nikon fluorescent microscope.

[1] HEK 293T/17 cells are loosely adherent. When adding or removing liquid try not to disturb the cells.
[2] 5-CF is light sensitive and care should be taken with the compound and once the cells take up the compound to protect from light.

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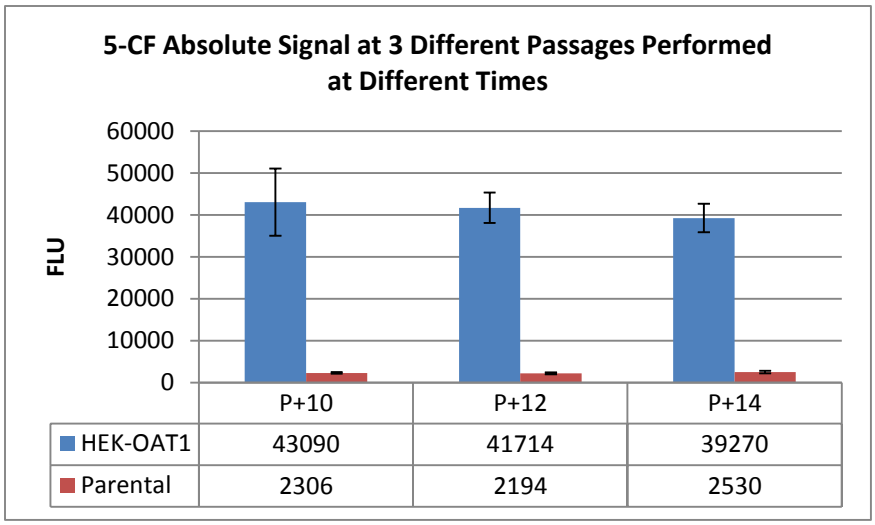

Figure1. Graph of OAT1 HEK 293T/17 absolute 5-CF signal over three passages.
OAT1 HEK 293T/17 and HEK 293T/17 absolute signal of 5-CF after lysis and monitored on a Promega GlowMax over three different passages.

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