RESOLUTE

Functional Assays for Solute Carrier Transporters

Membrane potential based assay for SLC13A3 using HEK-293 SLC13A3 OE cells

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Assay description

FLIPR® membrane potential dye measures changes of charges across the cell membrane, upon activation of SLC13A3. The assay allows the detection of ion channel and transporter modulation by increasing or decreasing the fluorescent signal as cellular membrane potential changes. When cells are depolarized dye enters the cells, causing an increase in fluorescent signal, conversely, cells hyperpolarization results in dye exit and decreased fluorescence (Figure 1).
SLC13A3 is the human Na^+/dicarboxylate cotransporter that couples 3 Na^+ ions to the transport of each divalent anion substrate. It transports a broad range of substrates including dicarboxylates containing from four to six carbon atoms (e.g., succinate), as well as tricarboxylates (e.g., citrate).
It is an electrogenic transporter eligible to be studied using the membrane potential dye.

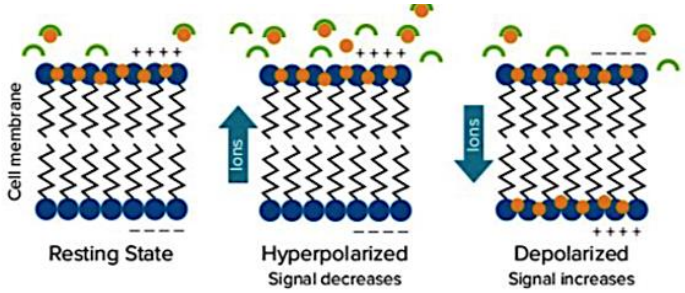


Figure 1. Principal of a FLIPR® membrane potential dye assay*. The assay measures changes of charges across the cell membrane, consequence of channels and transporters modulation. The fluorescent signal increases in intensity during membrane depolarization as dye follows the positively charged ions inside the cell. During membrane hyperpolarization, fluorescent signal decreases in intensity as dye follows the positively charged ions out of the cell.*

Assay protocol

HEK-293 JumpIN-SLC13A3 cells were subjected to pharmacological characterization.

Cell preparation
Cells were detached from 80-90% confluent flasks and seeded at 10'000 cells/well in black-clear bottom poly-D-Lysine coated 384 well plate in medium without the selective antibiotics and incubated 24 hours at 37°C, 5% CO_2. At the same time of seeding cells were induced with 1 µg/mL Doxycycline (not induced control in parallel).

Membrane Potential assay
Medium was removed and cells were incubated 30 minutes at RT in 20 µL/well of FMP-Blue-Dye (0.5X dye dissolved in Tyrode's Buffer Na^+ free as indicated in the manufacturer manual) and plates analysed at FLIPR^{TETRA} reader using a λexc 510 - 545nm / λem 565 - 625 nm filter.
To test pharmacology 20 µL/well of substrates: succinic acid disodium salt (2X in Standard Tyrode's Buffer), starting from 3 mM, semi-log dilution steps (8 concentrations, only buffer included), citric acid (2X in Standard Tyrode's Buffer), starting from 10 mM, 1:2 dilution steps (8 concentrations, only buffer included), a-ketoglutaric acid (2X in Standard Tyrode's Buffer), starting from 1 mM, semi-log dilution steps (8 concentrations, only buffer included) were online injected at the plate reader and fluorescence recorded.

Data analysis
FLIPR^{TETRA} measurements obtained from different well replicates were analysed by using the Screenworks software (Molecular Devices, Version 3.0.1.4). Absolute Response (RFU) is obtained applying "Subtract Bias on Sample: n" (where n = Timepoint of compound injection) whereas Assay Window Response (ΔF/F0) is obtained applying "Response over Baseline" correction (where Baseline = Timepoint -1 and -2 before activator injection) and "Background Subtraction". Data were then exported as Area Under the Curve (AUC). Mean and standard deviation of each replicate were calculated on the exported data with Excel software, then values were used to create sigmoidal dose-response curves (variable slope) and to calculate EC50/IC50 values with

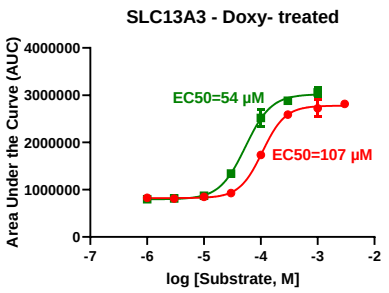
GraphPad PRISM software (Version 6).

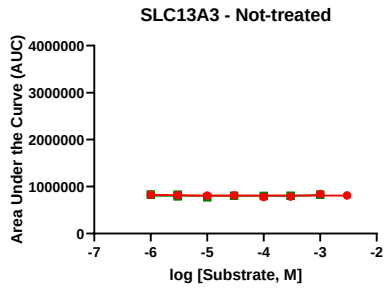
Additional information

Target data

SLC	SLC13A3
Synonyms	Na(+)/Dicarboxylate Cotransporter 3; NADC3
SLC sub-family	Solute Carrier Family 13 (Sodium-Dependent Dicarboxylate Transporter)
UniProt ID	Q8WWT9
RESOLUTE Cell ID	CE025V-1(HEK-SLC13A3-WTOE-p5-6)

Assay data





Compound name (1)	Succinic acid
PubChem CID	1110
Vendor (catalogue #)	Sigma Aldrich, # 224731
Mode of action	Substrate
Standard value type (i.e EC50, PoC, etc)	EC_{50} = 107 µM
Compound name (2)	a-ketoglutaric acid
PubChem CID	164533
Vendor (catalogue #)	Sigma Aldrich, # 75890
Mode of action	Substrate
Standard value type (i.e EC50, PoC, etc)	EC_{50} = 54 µM

Discussion

The Membrane Potential Assay for SLC13A3 showed a strong, specific and dose-dependent fluorescent signal upon injection of increasing doses of succinic acid and a-ketoglutaric acid. No response was detected upon injection of citric acid.

Cross references

- RESOLUTE report at Zenodo.