

# Membrane potential based assay for SLC1A1 using HEK-293 SLC1A1 OE cells

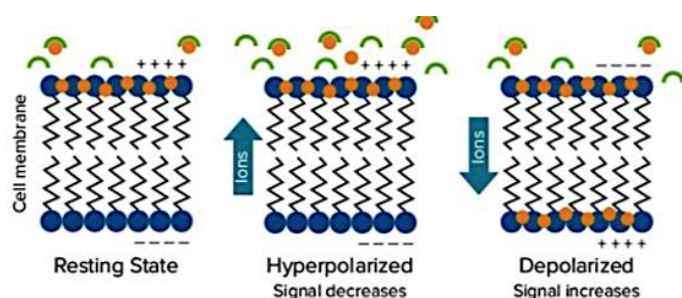
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## Assay description

FLIPR<sup>®</sup> membrane potential dye measures changes of charges across the cell membrane, upon activation of SLC1A1. 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).

SLC1A1 is a sodium-dependent, high-affinity amino acid transporter that mediates the uptake of L-glutamate and L-aspartate/D-aspartate. It is also reported to mediate the transport of L-cysteine. It functions as symporter that transports one amino acid molecule together with two or three Na<sup>+</sup> ions, thus it is an electrogenic transporter eligible to be studied using the membrane potential dye.



**Figure 1. Principal of a FLIPR<sup>®</sup> 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-SLC1A1 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<sub>2</sub>. At the same time of seeding cells were induced with 1 µg/mL Doxycycline (not induced and mock 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 Standard Tyrode's Buffer as indicated in the manufacturer manual) and plates analysed at FLIPR<sup>TETRA</sup> reader using a λ<sub>exc</sub> 510 - 545nm / λ<sub>em</sub> 565 - 625 nm filter.

To test pharmacology 20 µL/well of substrates: L-Cysteine; L-Glutamic acid; L-Aspartic acid (2X in Standard Tyrode's Buffer), starting from 3 mM, semi-log dilution steps (8 concentrations, only buffer included) were online injected at the plate reader and fluorescence recorded.

### Data analysis

FLIPR<sup>TETRA</sup> 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/F<sub>0</sub>) is obtained applying "Response over Baseline" correction (where Baseline = Timepoint -1 and -2 before activator injection) and "Background Subtraction". Data were then exported as Maximum (MAX). 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 EC<sub>50</sub>/IC<sub>50</sub> values with GraphPad PRISM software (Version 6).

## Additional information

## Target data

SLC	SLC1A1
Synonyms	Excitatory Amino Acid Transporter 3, EAAT3
SLC sub-family	Solute Carrier Family 1 (Glial High Affinity Glutamate Transporter)
UniProt ID	P43005
RESOLUTE Cell ID	CE0291-P (HEK-SLC1A1-WTOE-p5-6)

## Assay data

<p><b>L-Cysteine</b> MP dye incubation in Tyrode's standard</p>	<b>Compound name (1)</b>	L-Cysteine
	PubChem CID	5862
	Vendor (catalogue #)	Sigma Aldrich, # W326305
	Mode of action	Substrate
	Standard value type (i.e EC50, PoC, etc)	EC <sub>50</sub> = 161 μM
<p><b>L-Glutamic acid</b> MP dye incubation in Tyrode's standard</p>	<b>Compound name (2)</b>	L-Glutamic acid
	PubChem CID	33032
	Vendor (catalogue #)	Sigma Aldrich, # G1251
	Mode of action	Substrate
	Standard value type (i.e EC50, PoC, etc)	EC <sub>50</sub> = na The compound is eliciting a specific response, but a broader concentration range is needed, to calculate a correct EC50.
<p><b>L-Aspartic acid</b> MP dye incubation in Tyrode's standard</p>	<b>Compound name (3)</b>	L-Aspartic acid
	PubChem CID	5960
	Vendor (catalogue #)	Sigma Aldrich, # A9265
	Mode of action	Substrate

	Standard value type (i.e EC50, PoC, etc)	EC <sub>50</sub> = na The compound is eliciting a specific response, but a broader concentration range is needed, to calculate a correct EC50.
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## Discussion

The Membrane Potential Assay for SLC1A1 showed a specific and dose-dependent fluorescent signal upon injection of increasing doses of L-Cysteine. L-Glutamic Acid and L-Aspartic Acid induced a slight but specific fluorescent response in the target cell line.

## Cross references

- RESOLUTE report at [Zenodo](#).