RESOLUTE

Functional Assays for Solute Carrier Transporters

Membrane potential based assay for SLC22A4 using HEK-293 SLC22A4 OE cells

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Assay description

FLIPR® membrane potential dye measures changes of charges across the cell membrane, upon activation of SLC22A4. 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). SLC22A4 is an organic cation transporter that mediates Ergothioneine transport with a stoichiometry of two sodium per Ergothioneine transported, thus determining dye signal increase upon activation.

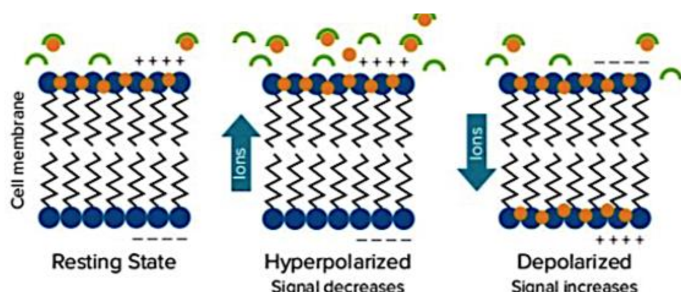


Figure 1. Principal of a FLIPR® membrane potential dye assay*. The assay measures changes of charges across the cell membrane, consequence of channels and transporters modulation. The fluorescent signal increases in intensity during membrane depolarization as dye follows the positively charged ions inside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

Assay protocol

To develop a functional recombinant SLC22A4 cell-based assay, HEK-293 cells were stably transfected with the SLC22A4 coding sequence. Mock control (transfected with the empty plasmid) was generated in parallel. Two rounds of limiting dilution led to the isolation of pure clones, the final clone was subjected to pharmacological characterization and tested to verify that the assay fulfils HTS quality criteria in terms of robustness and reproducibility.

Cell preparation
Cells were detached from 80-90% confluent flasks and seeded at 20’000 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.

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 Hamamatsu FDSS7000EX reader using a λexc 531nm/λem 593 nm filter (or at FLIPR^{TETRA} reader using a λexc 510 - 545nm / λem 565 - 625 nm filter).
To test pharmacology 10 µL/well of Ergothioneine D/R (3X in Standard Tyrode’s Buffer; 0.5% DMSO final concentration), starting from 500 µM, 1:3 dilution steps (8 concentrations, only buffer included) was online injected at the plate reader.
To assess robustness, reproducibility and amenability to high-throughput screening three 384-well plates were run consecutively, using the following plate layouts for one (Figure 2) or double injection protocol (Figure 3) respectively.

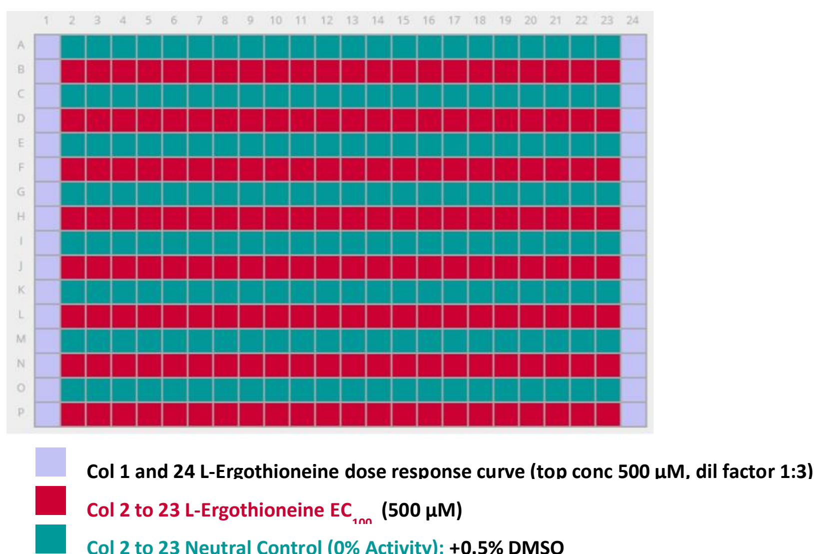


Figure 2. Compound plate layout for single injection protocol.

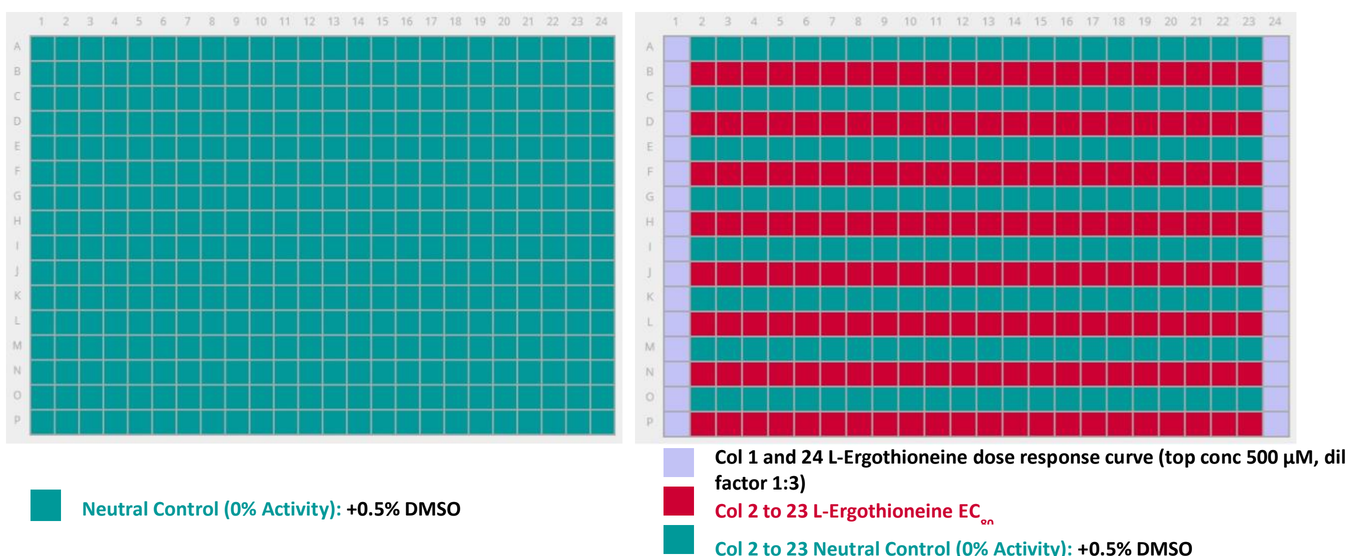


Figure 3. Compound plate layout for double injection protocol*. First injection plate on the left and second injection plate on the right.*

Data analysis

Hamamatsu FDSS7000EX measurements were analysed by using the Hamamatsu software (FDSS7000EX/µCELL software U8524-03A). Absolute Response (Relative Fluorescence unit, RFU) was obtained applying "Subtract Bias on Sample: n" (where n = Timepoint of compound injection). 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 8).

Additional information

Target data

SLC	SLC22A4
Synonyms	Organic Cation/Carnitine Transporter 1, OCTN1
SLC sub-family	Solute Carrier Family 22 (Organic Cation/Zwitterion Transporter)
UniProt ID	Q9H015
RESOLUTE Cell ID	HEK-293/SLC22A4 K1.3 p4 (Axxam cell line)

Assay data

Compound name (1)	L-Ergothioneine
PubChem CID	5351619
Vendor (catalogue #)	Santa Cruz Biotechnology, #sc-200814
Mode of action	Substrate
Standard value type (i.e EC50, PoC, etc)	EC_{50} = 40 µM

Z' factor calculated as

$$RZ' = 1 - \frac{3 \cdot RSD(CR_p) + 3 \cdot RSD(SR_p)}{|\langle CR_p \rangle - \langle SR_p \rangle|}$$

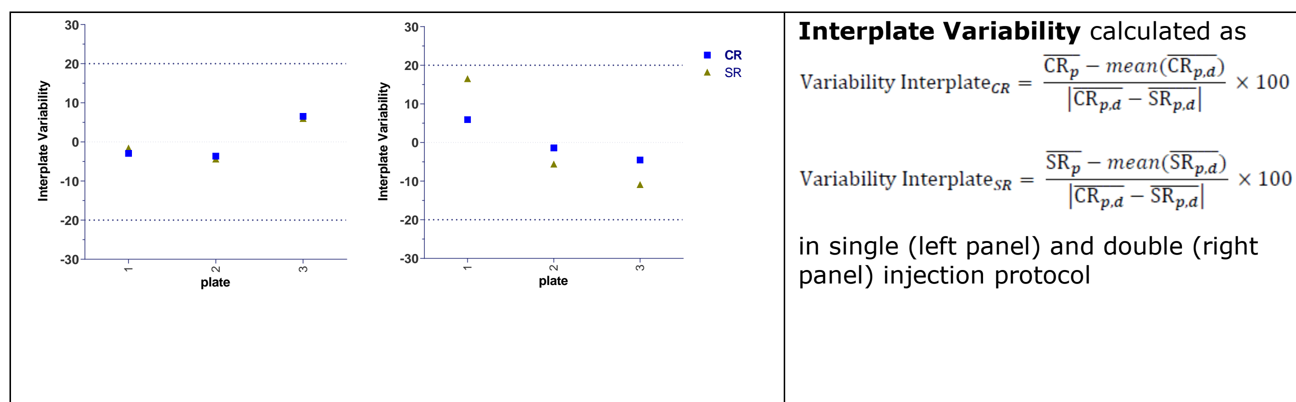
in single (left panel) and double (right panel) injection protocol

Intraplate Variability calculated as

$$\text{Variability Intraplate}_{CR} = \frac{SD(CR_p)}{|SR_p - CR_p|} \times 100$$

$$\text{Variability Intraplate}_{SR} = \frac{SD(SR_p)}{|SR_p - CR_p|} \times 100$$

in single (left panel) and double (right panel) injection protocol



Interplate Variability calculated as

$$\text{Variability Interplate}_{CR} = \frac{\overline{\mathrm{CR}_p} - mean(\overline{\mathrm{CR}_{p,d}})}{|\overline{\mathrm{CR}_{p,d}} - \overline{\mathrm{SR}_{p,d}}|} \times 100$$

$$\text{Variability Interplate}_{SR} = \frac{\overline{\mathrm{SR}_p} - mean(\overline{\mathrm{SR}_{p,d}})}{|\overline{\mathrm{CR}_{p,d}} - \overline{\mathrm{SR}_{p,d}}|} \times 100$$

in single (left panel) and double (right panel) injection protocol

Discussion

The Membrane Potential Assay for SLC22A4 showed a dose-dependent cell depolarization upon L-Ergothioneine injection.

The assay fulfils the HTS quality criteria in terms of RZ' and intraplate and interplate variability in both single and double injection protocols.

Cross references

- RESOLUTE report at Zenodo.