RESOLUTE

Functional Assays for Solute Carrier Transporters

Membrane potential based assay for SLC6A8 using HEK-293 JumpIN SLC6A8 OE cells

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Assay description

FLIPR® membrane potential dye measures changes of charges across the cell membrane, upon activation of SLC6A8. 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). SLC6A8) is a Na^+/Cl^- coupled electrogenic cotransporter, with a 2 Na^+:1 Cl^-:1 creatine stoichiometry, thus determining dye signal increase upon activation.
It mediates creatine uptake into a variety of cells, including neuronal cells, skeletal and cardiac muscle cells and intestinal and kidney epithelial cells and it is important in the maintenance of ATP homeostasis in tissues that have high energy requirement.

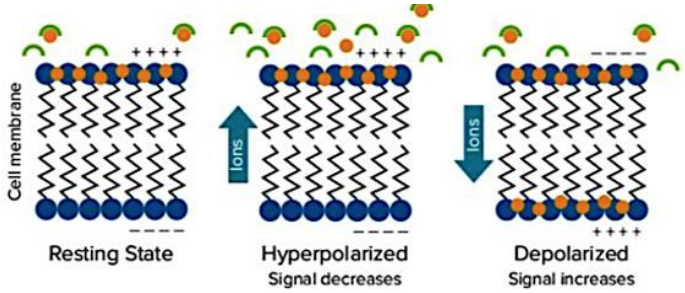


Figure 1. Principal of a FLIPR® membrane potential dye assay*. The assay measures changes of charges across the cell membrane, consequence of channels and transporters modulation. The fluorescent signal increases in intensity during membrane depolarization as dye follows the positively charged ions inside 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 SLC6A8 cell-based assay, HEK-293 JumpIN-SLC6A8 cells were 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. Cells were overnight 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 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 Creatine D/R (3X in Standard Tyrode's Buffer; 0.5% DMSO final concentration), starting from 5 mM, 1:10 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 or four 384-well plates were run consecutively using the following plate layouts for one (Figure 2) or double injection protocol (Figure 3) respectively.

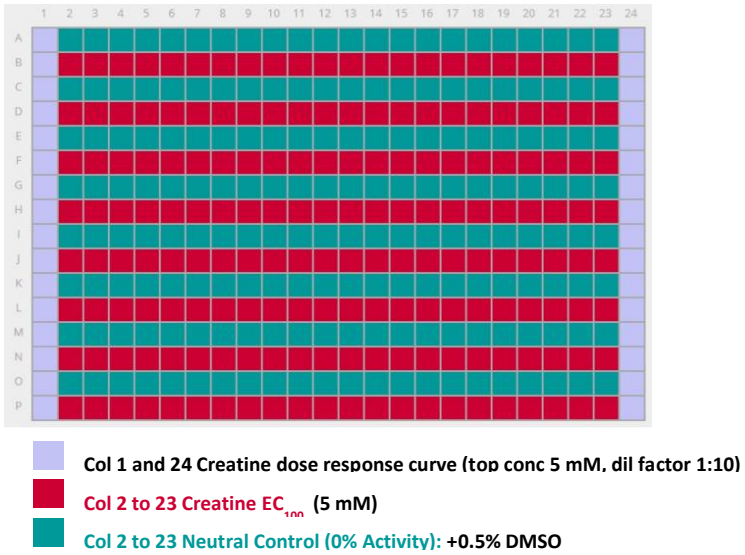


Figure 2. Compound plate layout for single injection protocol.

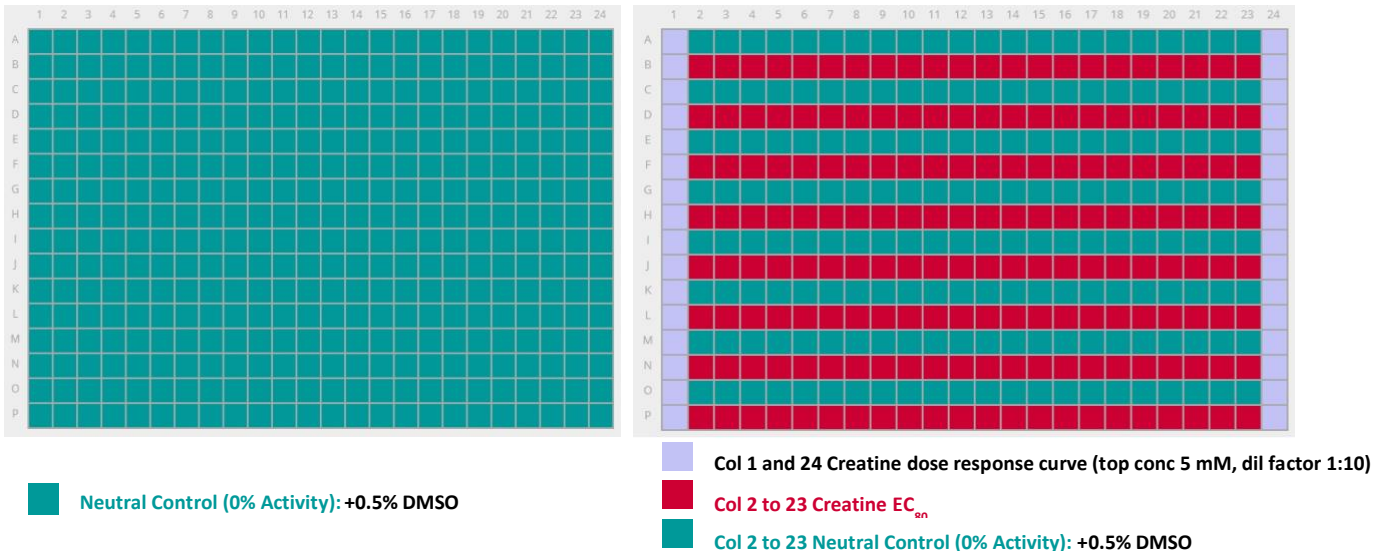


Figure 3. Compound plate layout for double injection protocol*. First injection plate on the left and second injection plate on the right.*

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).

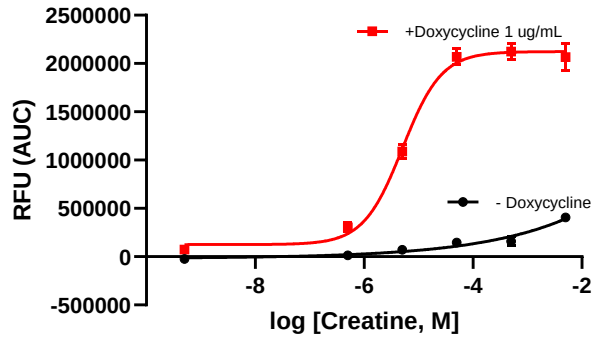
Mean and standard deviation of each replicate were calculated on the exported data with Excel software, then values were used to create sigmoidal dose-response curves (variable slope) and to calculate EC50/IC50 values with GraphPad PRISM software (Version 8).

Additional information

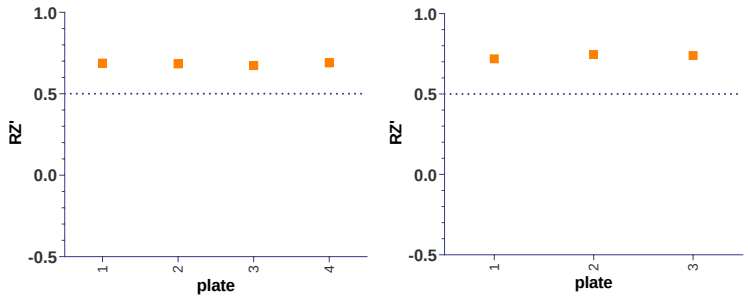
Target data

SLC	SLC6A8
Synonyms	Sodium- And Chloride-Dependent Creatine Transporter 1, CRTR
SLC sub-family	Solute Carrier Family 6 (Neurotransmitter Transporter)
UniProt ID	P48029
RESOLUTE Cell ID	CE02KH-M (HEK-SLC6A8-WTOE-p3)

Assay data



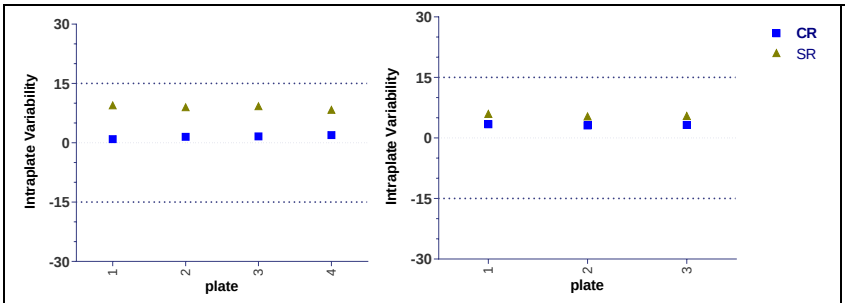
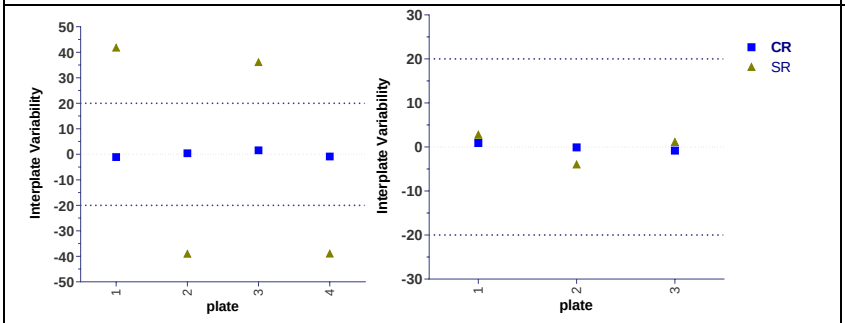
Compound name (1)	Creatine
PubChem CID	586
Vendor (catalogue #)	Sigma, #C0780
Mode of action	Substrate
Standard value type (i.e EC50, PoC, etc)	EC_{50} = 5 μM



Z' factor calculated as

$$RZ' = 1 - \frac{3 \cdot RSD(\mathrm{CR}_p) + 3 \cdot RSD(SR_p)}{\left|\langle \mathrm{CR}_p \rangle - \langle \mathrm{SR}_p \rangle\right|}$$

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

	**Intraplate Variability** calculated as $\text{Variability Intraplate}_{CR} = \frac{SD(\text{CR}_p)}{\lvert\overline{\text{SR}_p} - \overline{\text{CR}_p}\rvert} \times 100$ $\text{Variability Intraplate}_{SR} = \frac{SD(\text{SR}_p)}{\lvert\overline{\text{SR}_p} - \overline{\text{CR}_p}\rvert} \times 100$ in single (left panel) and double (right panel) injection protocol
	**Interplate Variability** calculated as $\text{Variability Interplate}_{CR} = \frac{\overline{\text{CR}_p} - mean(\overline{\text{CR}_{p,d}})}{\lvert\overline{\text{CR}_{p,d}} - \overline{\text{SR}_{p,d}}\rvert} \times 100$ $\text{Variability Interplate}_{SR} = \frac{\overline{\text{SR}_p} - mean(\overline{\text{SR}_{p,d}})}{\lvert\overline{\text{CR}_{p,d}} - \overline{\text{SR}_{p,d}}\rvert} \times 100$ in single (left panel) and double (right panel) injection protocol

Discussion

The Membrane Potential Assay for SLC6A8 showed a dose-dependent cell depolarization upon Creatine injecton.

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.