RESOLUTE — Research Empowerment on Solute Carriers

Selection of the most appropriate technology to develop an HTS grade SLC assay

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SLC assay development for screening purpose

The development of SLC functional assays for running High-throughput screening campaigns represents a fundamental approach to discover novel inhibitors and activators of SLC transporters for therapeutic purposes.

Different screening strategies have been used for the discovery of SLC modulators, including cellular substrate uptake assays with radioactivity or fluorescence detection; downstream functional assays with methods based on detection of pH and membrane potential; ligand binding assays with detection methods including radioactivity, fluorescence, mass spectrometry, or thermal shift (CETSA); phenotypic screening assays with different target identification methods.

In vitro, cell-free methods can also be used, anyway due to the SLC complex topography and numerous transmembrane domains, SLCs often require conditions that mimic their native environment to remain properly structured and functionally active; that's way cellular systems are preferable for the development of assays for screening purpose.

In the context of Resolute WP3 and WP6, Axxam has contributed, by mean of its consolidated experience and expertise, in the development of functional assays for many different SLCs, applying multiple and diversify technologies including fluorescent dyes, genetically encoded sensors, fluorescent substrate-based detection methods.

Assay requisites for screening purpose

To be suited for running a High Throughput Screening (HTS), an assay, for a cell line recombinantly expressing the SLC of interest, must satisfy some fundamental quality parameters:

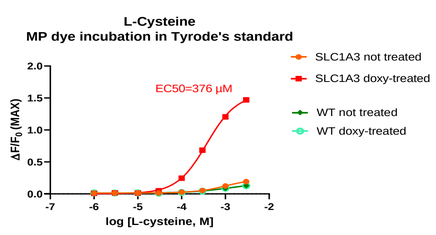
- Possibility of miniaturization at least in 384 well plate format
- Specific response to the substrate (native or surrogate)
- Negligible response in mock transfected or non-induced cells
- Significant signal to background window, displaying a proper RZ' factor, which considers the signal amplitude and the standard deviation
- Signal stability over time
- Pharmacology reproducibility over time

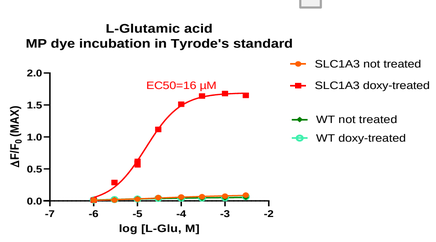
Some examples

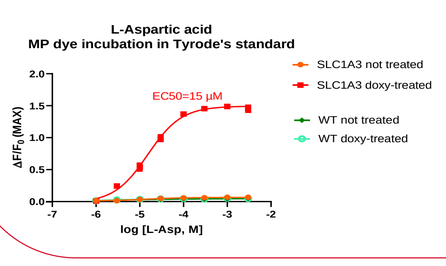
In this poster, some case studies of cell-based SLC assays, suitable for running HTS and developed by using different tools are shortly shown.

The detection systems used include Membrane Potential (MP) dye, SLC fluorescent substrate, Chloride and pH biosensors, Thallium dye, to record Thallium influx, as a surrogate for Potassium influx.

HEK293 CELL LINE STABLY EXPRESSING SLC1A3 IN INDUCIBLE SYSTEM, VS. WT AND NON-INDUCED CELLS

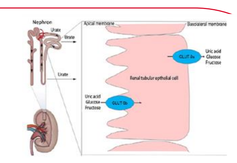


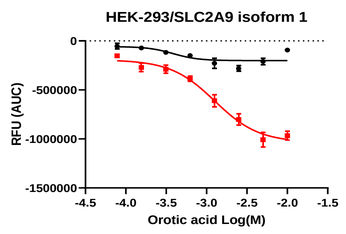


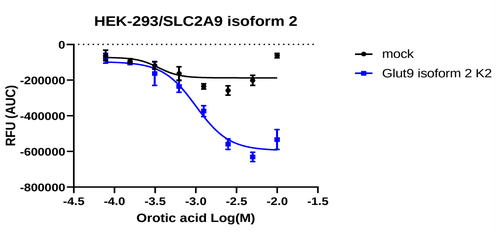


A specific and substrate dose-dependent fluorescent response, due to the influx of Na+, was detected only in the SLC1A3 induced condition, using **Membrane Potential dye**

HEK293 CELL LINE STABLY EXPRESSING SLC2A9 IN CONSTITUTIVE SYSTEM, VS. WT CELLS





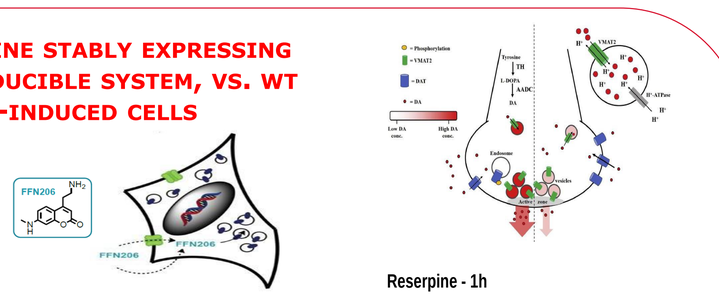


Dose-response of the negatively charged substrate **Orotic acid** in HEK-293/SLC2A9 isoform 1 (**EC50 ~ 1.5 mM**), recorded as fluorescence decrease signal.

Dose-response of the negatively charged substrate **Orotic acid** n HEK-293/SLC2A9 isoform 2 (**EC50 ~ 1 mM**), recorded as fluorescence decrease signal.

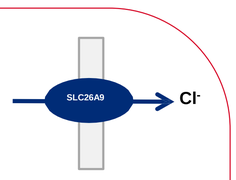
Using **Membrane Potential dye**, a significant hyperpolarization was obtained, upon SLC stimulation, in both isoforms of target cells; it was almost undetectable in mock transfected cells

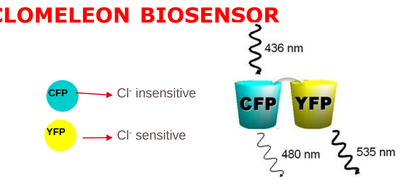
HEK293 CELL LINE STABLY EXPRESSING SLC18A2 IN INDUCIBLE SYSTEM, VS. WT AND NON-INDUCED CELLS

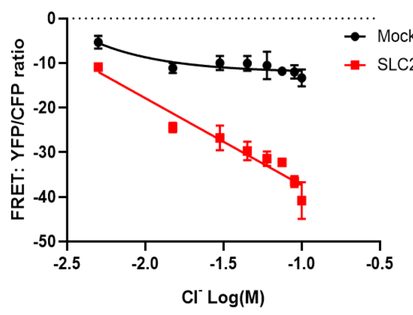


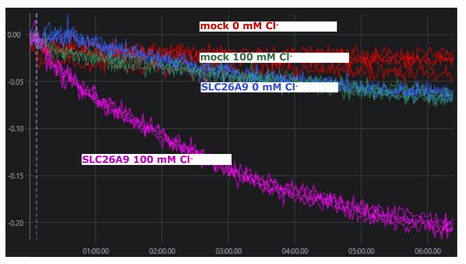
Stimulation of induced cells with the **fluorescent substrate FFN206** showed a dose/dependent fluorescence increase, with an EC50 value of ~ 2 µM
The response was dose dependently inhibited by reference inhibitors.
No fluorescence signal modulation was recorded in wt and uninduced cells

HEK293 CELL LINE STABLY EXPRESSING SLC26A9 AND TRANSIENTLY EXPRESSING THE SUPERCLOMELEON BIOSENSOR





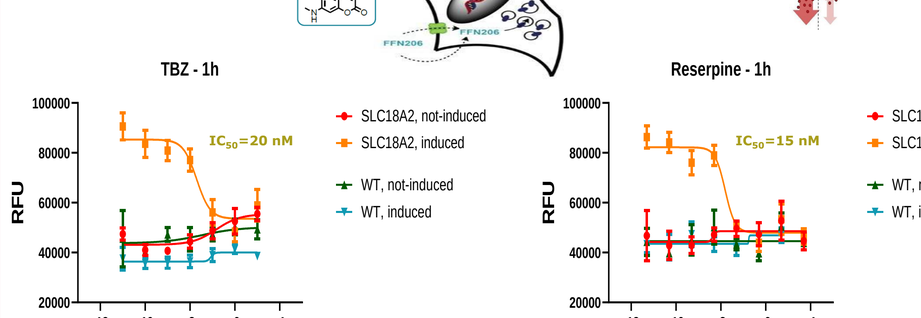




Fluorescent kinetic traces

A significant FRET signal decrease of the **Superclomeleon sensor** was detected only in the SLC26A9 cells, and not in mock transfected cells, upon stimulation with Chloride dose response

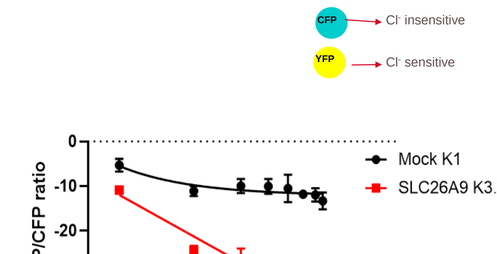
HEK293 CELL LINE STABLY EXPRESSING SLC16A3, IN INDUCIBLE SYSTEM, AND TRANSIENTLY EXPRESSING THE PHAXSENSOR

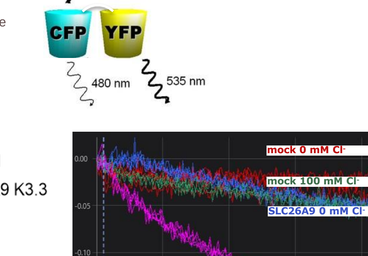


Both the red and green fluorescence recordings, of the pHAxsensor, are shown.
The red fluorescence signal resulted unchanged, due to the pH-insensitivity of this moiety of the biosensor; in contrast the **green fluorescence, which is pH sensitive, is quenched** upon transporter activation.
Very weak signal was detected in uninduced cells (data not shown), due to endogenous expression of lactate transporters

HEK293 CELL LINE STABLY EXPRESSING SLC12A2 IN INDUCIBLE SYSTEM, VS. NON-INDUCED CELLS

Tl+ fluorescent kinetic traces





A dose response of Thallium was recorded in induced vs non-induced cells, with the **Tl+ sensitive dye**, showing an EC50 of 0.6 nM.
The signal, which was very weak in uninduced cells, was specifically inhibited by an SLC blocker.

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