Development of a High-Throughput Screening Compatible SLC6 Transporter Assay Measuring Sodium Flux

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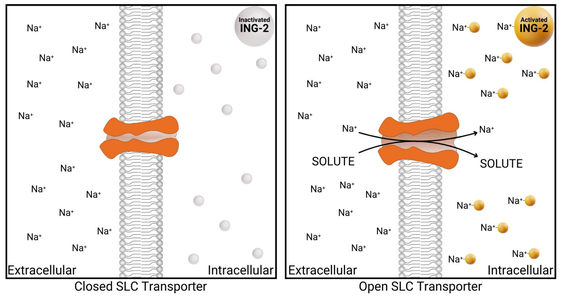
Abstract

- **Sodium-dependent solute carrier (SLC) transporters** are integral membrane proteins that mediate solute movement across cell membranes by coupling transport to sodium flux along the electrochemical gradient. The sodium-dependent SLC6 family plays a critical role in neurotransmission, with dysfunction linked to various neurological and psychiatric disorders.
- We developed a real-time functional assay to evaluate modulators of SLC6A1 (GABA transporter) and SLC6A9 (glycine transporter) using ION Biosciences' **Sodium-Dependent SLC Transporters Assay Kit**. This kit features **ION Natrium Green-2 AM (ING-2 AM)**, a sodium-sensitive fluorescent indicator that directly detects sodium flux associated with solute uptake. As solutes are transported across the membrane, extracellular sodium is co-transported into the cell, increasing ING-2 fluorescence. This provides a powerful tool for assessing the pharmacology of SLC transport modulators.
- The assay demonstrated a **Z' factor > 0.7**, indicating high quality and reliability for **high-throughput screening** (HTS) applications. These results support its suitability for drug discovery programs targeting SLC6 transporters.



Methods

1. Cell Seeding: HEK293T cells expressing SLC6A1 or SLC6A9 were seeded into 384-well plates at 10,000 cells per well, 24 hours before the assay.
2. Dye Loading: ING-2 AM was dissolved in a solution containing 1X SLC Sodium Assay Buffer, 1X DySolv, 1X Probenecid, and 1X TRS. The culture medium was replaced with 20 µL per well of this solution, followed by incubation at 37°C for 60 minutes.
3. Compound Incubation: A 3X concentrated modulator solution (NNC-711 for SLC6A1 or ALX-5407 for SLC6A9) was prepared and added to the cells (10 µL per well), followed by incubation at 37°C for 15 minutes.
4. Substrate Stimulation: A 4X concentrated substrate solution (GABA for SLC6A1 or glycine for SLC6A9) was prepared and added to the cells (10 µL per well) following a 30-second baseline fluorescence acquisition using Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm).
5. Data Analysis: Fluorescence data were baseline normalized (F/F_0), and the slope (V_{max}) was calculated from the first 30 seconds following stimulation.

Results

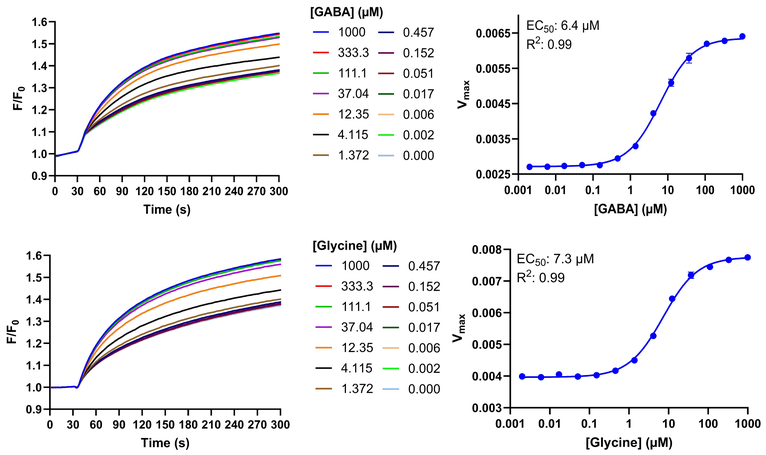


Figure 1. Solute Uptake Assay. HEK293T cells expressing SLC6A1 or SLC6A9 were treated with increasing concentrations of GABA or glycine following a 30-second baseline read. Uptake was measured, and data are presented as mean ± SEM (n = 3). The calculated EC_{50} values were 6 µM for GABA (SLC6A1) and 7 µM for glycine (SLC6A9).

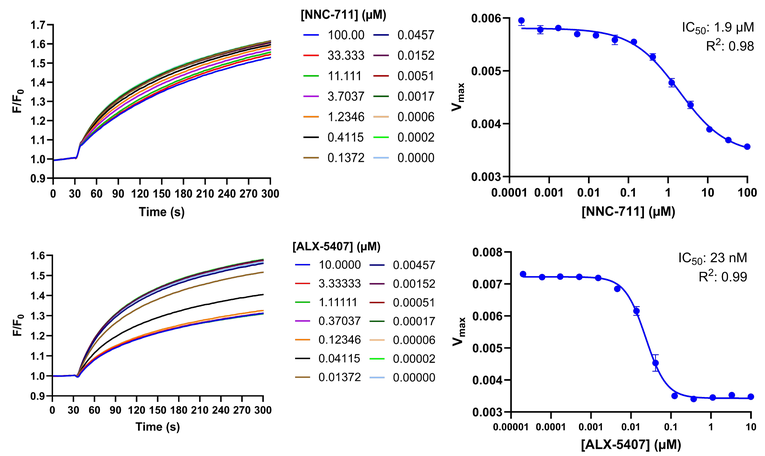


Figure 2. Inhibition of Solute Uptake Assay. HEK293T cells expressing SLC6A1 or SLC6A9 were pretreated with increasing concentrations of NNC-711 or ALX-5407 for 15 minutes before the assay. Following a 30-second baseline read, cells were treated with 1 mM GABA or 1 mM glycine. Uptake was measured, and data are presented as mean ± SEM (n = 3). The calculated IC_{50} values were 1.9 µM for NNC-711 (SLC6A1) and 23 nM for ALX-5407 (SLC6A9).

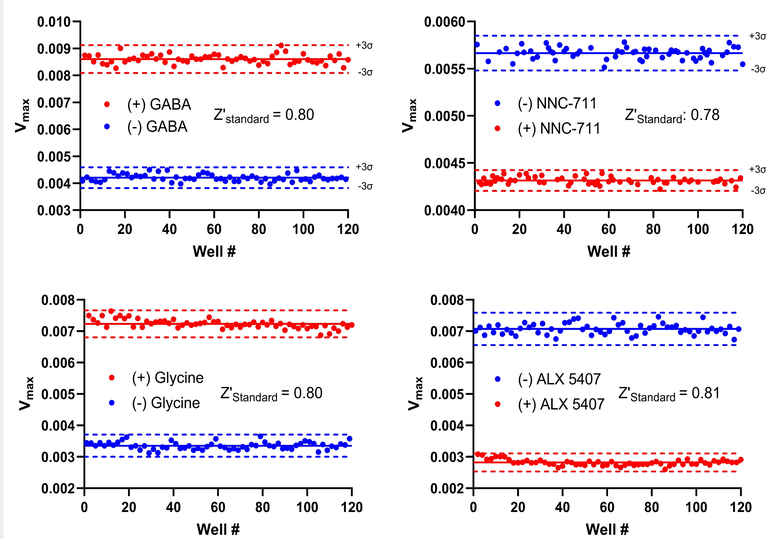


Figure 3. Z' Factor Determination. HEK293T cells expressing SLC6A1 or SLC6A9 were pretreated with or without 100 µM NNC-711 or 10 µM ALX-5407 for 15 minutes before the assay. Following a 30-second baseline read, cells were treated with or without 1 mM GABA or 1 mM glycine. Z' factor plots indicate Z' > 0.7 (n = 60). A Z' factor above 0.5 is considered an excellent assay.

Conclusions

- The **Sodium-Dependent SLC Transporters Assay Kit** exhibited excellent performance with a **Z' factor > 0.7**, demonstrating its high quality and suitability for HTS. The calculated EC50 values were 6 µM for GABA (SLC6A1) and 10 µM for glycine (SLC6A9), while the IC50 values were 1.9 µM for NNC-711 (SLC6A1) and 23 nM for ALX-5407 (SLC6A9). These findings highlight the assay's sensitivity and ability to effectively evaluate the pharmacology of transporter modulators.
- By utilizing the sodium-sensitive fluorescent indicator ING-2 AM to track sodium flux during solute uptake, this kit provides a robust and reliable platform for assessing SLC6A1 and SLC6A9 transporter activity. It is ideally suited for drug discovery efforts targeting SLC6 transporters, facilitating the identification of potential therapeutic agents for disorders associated with these transporters.