

Application of Molecular Devices Fluorescence Membrane Potential Sensitive Dye for the Study of a Human Electrogenic Transporter.

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Electronic transporters like GAT-3 are playing fundamental roles in the CNS. Therefore they are presenting also important target class for a drug intervention.

Here we are presenting data for the generation of a screening assay for GAT-3 based on the membran potential assay kit from Molecular Device.

HEK-293 cells either transiently or stably expressing the hGAT-3 were used to study the function and activity of the transporter both by electrophysiological (patch clamp) and fluorescence techniques.

We measured an inward current upon application of GABA, which was inhibited by the removal of external Na⁺.

The functionality of the hGAT-3 was equally investigated by applying α -alanin, a GAT-3 antagonist (in uptake studies), which induced a Na⁺ dependent inward current.

In FLIPR experiments with the MD membrane potential sensitive dye, addition of GABA induced increases in fluorescence in HEK-293 stably expressing hGAT3 in a dose-dependent manner.

Thus, the MD membrane potential dye could be a useful tool for developing high-throughput screening assays for electrogenic transporters.