Video Article

IonFlux: Automated Patch Clamp System with Plate Reader Simplicity - ADVERTISEMENT

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Abstract

Ion channels play a critical role in regulation of the central nervous system and the physiology of nerves and muscles. As a result they have been implicated in numerous disease conditions such as diabetes and epilepsy, and are leading targets for new medications. The patch clamp method is the gold standard for evaluating ion channel activity in vitro. When implemented in a manual fashion, the patch clamp assay is limited to around 10 data points per day, which is well below the threshold needed for meaningful drug discovery and development efforts. The IonFlux platform uses the proprietary Well Plate Microfluidic technology to automate the patch clamp method in a convenient and high throughput well plate format that comes in two scalable configurations to accommodate a broad range of applications, including both voltage- and ligand-gated ion channels. The IonFlux HT is suited for high throughput screening in drug discovery and development. This version is capable of producing up to 10,000 data points per day. For additional throughput, up to four IonFlux HT systems can be arrayed around a single liquid handling robot for up to 40,000 data points per day. The low cost per data point and minimal compound usage make the IonFlux HT ideal for drug screening, drug development, safety toxicology, and ion channel research. The IonFlux 16 is a cost-effective solution for moderate throughput applications such as cell line development, mutant screening, and assay development. For the first time ever, users have access to true automated patch clamp technology at a comparable cost to a manual patch clamp rig.

Video Link

The video component of this article can be found at http://www.jove.com/video/2392/

Protocol

1. IonFlux System Overview

1. The IonFlux System is comprised of a benchtop instrument, a proprietary microfluidic device in an SBS-standard well plate format, and a complete acquisition and analysis software package.
2. Unlike other automated patch clamp instruments, the IonFlux reader maintains a very small footprint which closely-resembles a plate reader. It easily fits on the benchtop, and has available integration with commonly-used laboratory automation robots, enabling the IonFlux to fit seamlessly into existing high throughput screening workflows as well as research laboratories.
3. The IonFlux utilizes ensemble recording, which means that a population of 20 cells are patched in each recording zone, and their currents are averaged to produce a single data point. This approach produces a high success rate and minimizes the effect of cell variability.
4. Each 384-well plate can process 256 unique compounds and produce 512 data points. The 96-well plate used with the IonFlux 16 processes 64 compounds and produces 128 data points.
5. Having the ability to run high throughput electrophysiology screens opens the door to a wide range of ion channel experiments. Common applications include experiments with ligand gated channels such as the GABA and nicotinic receptors, voltage gated channels such as the sodium and potassium channels, and safety toxicology focusing on the hERG potassium channel. Mutant and cell line screens can also be performed. Furthermore, the IonFlux enables analysis of the functional expression of ion channels.

2. Data Acquisition and Analysis

1. The IonFlux System comes with an advanced software package for data acquisition and analysis. Once the software is loaded, the user will see three primary screens: Setup, Runtime, and Data Analysis.
2. The Setup mode contains all of the controls needed to define an experiment. In this mode, experiments are initialized and whole or half plate is designated. The table of utilized compounds is also imported in set-up mode.
3. In the Experimental Sequence tab, parameters are set for the four phases of the experiment: Prime, Trap, Break, and Data Acquisition. Here, the voltage protocols are defined.
4. The Runtime mode shows the data as it is being collected. The Real-time Sweep Window displays currents generated during each voltage pulse for every channel. Experimental progress is also reported.

5. The Data Analysis mode enables quick review and analysis of completed experiments. Tools are provided for viewing sweep and trace data, as well as for logging specific events such as compound introduction. A Hit Map module helps identify the most promising compounds in the screen. All data can easily be exported in .csv format to Excel and other database applications.

3. IonFlux Applications - GABAA Modulators

1. A wide variety of experiments and target screens can be performed on the IonFlux System. Here, an example is demonstrated to assay for modulators of the human GABA-A receptor, expressed in HEK293 cells from Millipore. This ligand-gated chloride channel is involved in CNS inhibition, thus constituting an important drug target in epilepsy, anxiety, and insomnia.
2. To perform screening assays, cell ensembles were exposed to EC20 concentrations of the native ligand GABA, in the presence of ascending concentrations of modulator substances that bind at the allosteric site on the receptor.
3. The plots show dose-response curves for two positive allosteric modulators in the benzodiazepine class, namely triazolam and diazepam, for which EC50 values of 3 nM and 0.5 µM were obtained.
4. The modulator-dependent shift in apparent GABA affinity was then determined by cumulatively applying different GABA concentrations in the presence of a fixed concentration of the modulator, in this case, 3 µM diazepam.
5. This type of experiment is enabled by the flow-through fluidic design available in the IonFlux instruments. The EC50 for GABA was shifted by about 1 µM to lower concentrations due to increased binding affinity in the presence of the modulator.

4. IonFlux Applications - Blockers of Neuronal hNav1.8 Sodium Channel

1. The IonFlux Instrument also facilitates other pharmacological experiments, including the example shown here typical for blockers of the neuronal hNav1.8 sodium channel, a voltage-gated channel involved in the perception of pain. In this assay, the Na^+ channel, expressed in HEK293 cells was activated by voltage clamp steps from -120 mV to 0 mV, applied every second.
2. This protocol therefore provides information on tonic block of the channel, since the channel re-enters the rested state between depolarizations. Cumulative blocker dose-response curves are illustrated for the local anesthetics lidocaine and tetracaine, providing IC50 values of 320 µM and 34 µM, respectively.
3. In a second graph, the lidocaine affinity for hNaV1.8 is compared to that for hNaV1.7, another voltage-gated Na^+ channel involved in pain sensation. The IC50 for hNaV 1.7 was found to be 3 mM, revealing a ten-fold lower affinity when this channel binds lidocaine, compared with hNaV1.8.

5. IonFlux Applications - hERG Screening

1. The IonFlux instrument can also be used for safety pharmacology applications such as hERG screening. The hERG potassium channel has been implicated in adverse cardiac events associated with diverse drugs on the market, leading to their withdrawal; and it is now required that all new drug compounds be screened against this channel.
2. Blocker dose-response curves were performed for characteristic voltage-dependence hERG channels expressed in CHO cells for three commonly used reference compounds, namely terfenadine, cisapride and quinidine.
3. The graphs show the typical current profile obtained when hERG channels are activated by a two-pulse protocol, which is necessary because of its rapid inactivation rate, as used to reveal hERG tail currents. The graphs show the activation voltage-dependence and rectification properties of the hERG current recorded in the IonFlux instrument.
4. These recordings closely replicate accepted channel behavior represented in the literature. The blocker dose-response curves reveal IC50 values for the three reference compounds of 60 nM, 160 nM, and 1 mM.