Bringing plate reader simplicity
to ion channel screening

FLUXION


# IONFLUX™

High throughput automated patch clamp system

# IonFlux is the first automated patch clamp system which offers simplicity, affordability, and throughput in a single instrument.

The IonFlux System delivers a high throughput solution for ion channel drug discovery and research. The system offers outstanding patch clamp performance in a complete, easy to use system with “plate reader simplicity”. Fluxion’s proprietary Well Plate Microfluidic™ technology eliminates pipetting steps, and allows continuous recording of cell ensembles. The fast compound addition and continual washing enables both ligand-gated and voltage-gated studies.

***A system for every application***

IonFlux comes in two configurations to meet the widest variety of application needs. The IonFlux HT, featuring 64 independent amplifier channels, is designed for high throughput screening of ion channel targets. The IonFlux 16 is ideally suited for ion channel research and assay development. Both systems offer cell ensemble recording to improve reliability and variability.

# Ion channels are an important, yet under served drug target class, making up over one fourth of druggable targets

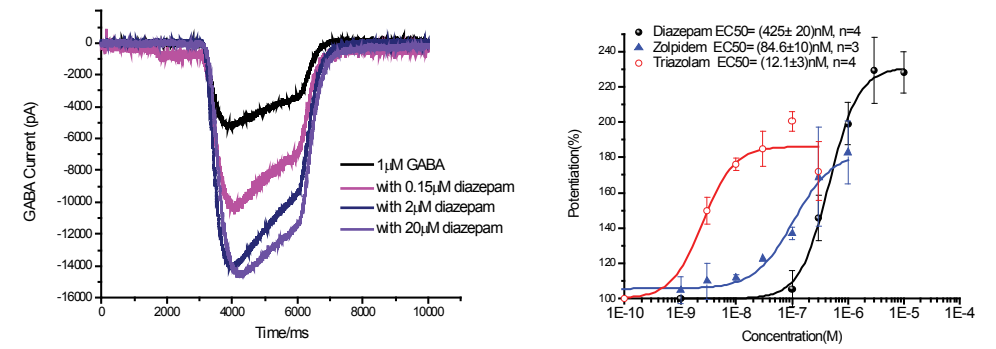
"Despite their attractiveness as drug discovery targets ion channels remain an under-exploited target class, which is in large part due to the labour-intensive and low-throughput nature of patch-clamp electrophysiology."

Dunlop, J. et al.
Nature Reviews Drug Discovery: 7, 358-368

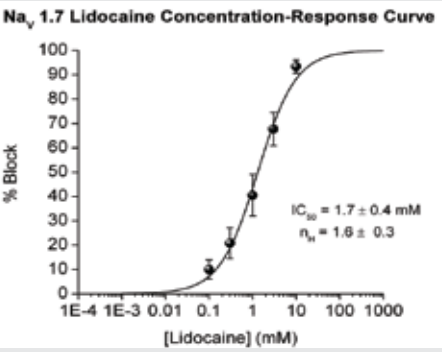
IonFlux is the first instrument of its kind to offer a **scalable solution** for automated electrophysiology. With a **low cost per data point, experimental flexibility, and space-saving footprint**, the IonFlux System is ready to meet all of your ion channel research and drug discovery needs.

## Pain and anxiety

**Modulation of the GABA response by diazepam and two other allosteric modulators**
Diazepam (valium) and other GABA positive allosteric modulators characterized using the IonFlux system. Pharmaceutical companies are developing this target class to improve treatment for mood disorders (i.e. anxiety) and chronic pain.




## Local anesthetics and antiarrhythmic compounds




**Sodium channel blockers like lidocane are being developed across a broad number of indications**
Data obtained with the IonFlux System exemplifies the functional characterization of the local anesthetic lidocane as an effective sodium channel blocker. Shown here, Nav 1.7 current suppressed by increasing compound concentrations.

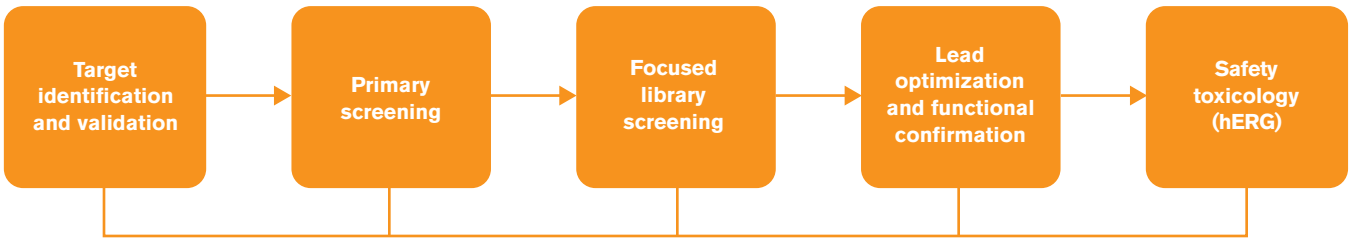
## In ion channel research…

Ion channels research is critical to understanding critical biological processes occurring throughout the cardiovascular, metabolic, and nervous systems. The manual patch clamp technique is often employed to record currents from individual cells, but these experiments are limited to smaller data sets. The IonFlux 16 System is the first instrument of its kind to offer the convenience and throughput of automated patch clamping, at a price that fits the individual research lab budget. IonFlux can be used for a wide variety of ion channel investigations, including:

- Functional expression of ion channels
- Compound profiling
- Mutant and cell line screening
- Recording from primary cells
- Transient transfection

## In ion channel drug discovery and development…

Ion channels represent a major classification of targets for drug discovery. They play critical roles in many disease conditions including pain, epilepsy, Alzheimer's, and cardiovascular conditions. Patch clamp measurements are essential to ion channel drug discovery, but the manual technique and first generation automated instruments do not provide the throughput and flexibility required to meet the needs of screening workflows and cost targets. The IonFlux HT System offers the highest throughput at the lowest running cost of any automated patch clamp instrument.




**IonFlux Application Areas**

| Features | Benefits |
|---|---|
| High throughput design with 16 or 64 channels of amplification run in parallel | Reduces time to publication<br>Accelerates drug discovery and development |
| Simple to use with "plate reader simplicity" | Eliminates need for manual patch clamp technique<br>Ensures quick setup of experiments |
| Small, bench top footprint<br>Compatible with lab automation equipment | Reduces lab space needed<br>Facilitates integration with existing HTS workflows |
| Addresses both ligand- and voltage-gated ion channels<br>Continuous recording capability with full wash-out | Ensures flexibility to address a wide variety of ion channel investigations and screening campaigns. |

# Principles of Operation

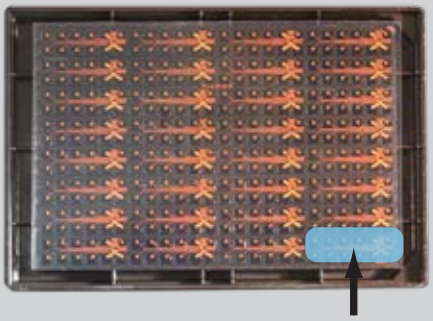
## Well Plate Microfluidics: Providing the best of both worlds

IonFlux utilizes the innovative Well Plate Microfluidic™ technology to embed micron-scale fluidic channels on the bottom of an SBS-standard well plate. This approach offers the convenience of using well plates with existing laboratory equipment. At the same time, it provides a high degree of functionality and flexibility for running ion channel investigations.

**1. Cells and compounds are added to the well plate.**

Each IonFlux Plate contains an array of experimental patterns. Each experimental pattern comprises 12 wells: 8 for compounds, 2 for cell trapping, and 2 for cell inlet and outlet.

Cells are loaded in the inlet well, buffer is loaded into the trapping wells, and 8 unique compounds or concentrations series are loaded into the remaining wells of the experimental pattern.



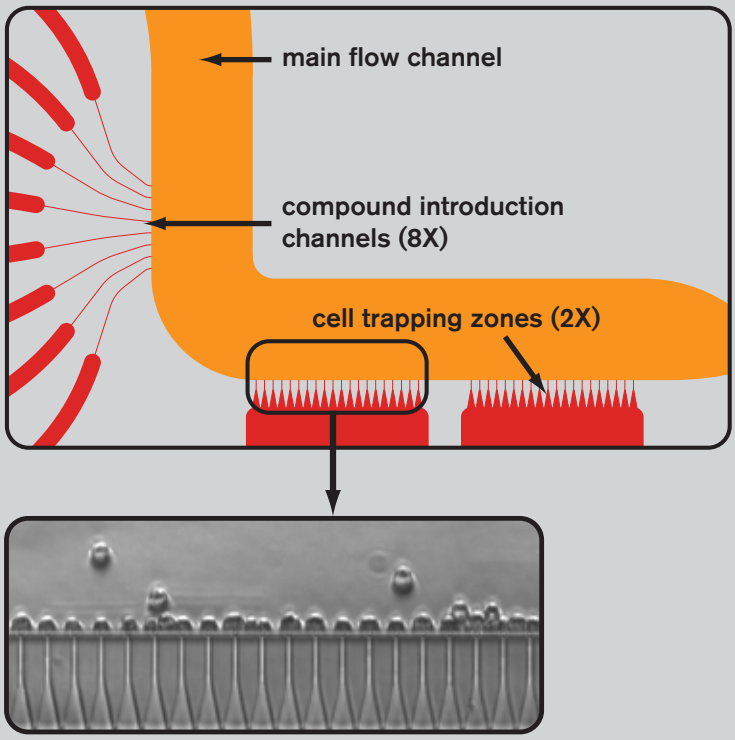

**2. Cells are trapped in an ensemble array.**

Cells are pushed through the main flow channel using pressure from the instrument. An ensemble of 20 cells is trapped in small channels which resemble patch pipettes. There are two ensembles of 20 cells per experimental pattern each of which gets exposed to the same group of 8 compounds. This provides 8 data points in duplicate for enhanced data fidelity.

**3. Current is recorded from each ensemble.**

Electrodes from the instrument are placed in fluidic contact with each 20 cell ensemble. Current is measured from the group of 20 cells using a discrete patch clamp amplifier. This approach produces high success rates since the recording averages the current across all 20 cells.

Compounds can be applied across the cells with full washout in between . The system uses continuous recording to facilitate recording from fast-acting ion channels.



# Key applications in drug discovery and research

## Ligand-gated ion channels

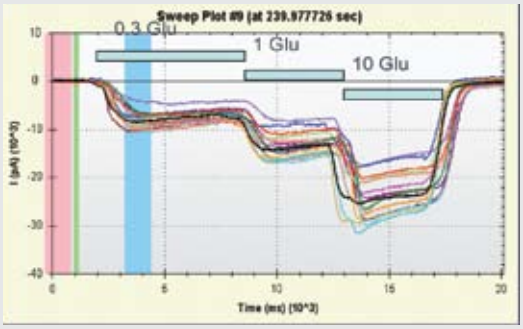
The microfluidic well plate technology employed by the IonFlux system enables unlimited flexibility and synchronous compound additions across the full plate. This leads to increased throughput (8 minutes per plate for LGIC targets, determined by compound incubation times). Continuous perfusion also dramatically improves ligand washout, enabling the study of challenging targets such the NMDA receptor.

**Key advantages for this application class:**

- Continuous perfusion
- Superior washout (>50x volume exchange per wash step)
- Limitless protocol flexibility (many repeated applications with no lag time between additions)
- Superior consistency of response due to ensemble recording

**Representative applications:**

- GABA receptor agonist screens
- NMDA receptor screens
- Nicotinic receptor studies
- P2X purinergic receptor pharmacology




Current response from the NMDA receptor is plotted as a function of increasing ligand concentrations. NMDA is a challenging assay because cell health is compromised by activation of the receptor. As a result, washout must be fast and complete. Data shown is enabled by a protocol unique to the IonFlux instrument: three consecutive agonist concentrations are applied without wait times in between, bracketed by wash buffer. An EC50 is assembled from each ensemble in order to assess shifts in response to compound modulation.

## Voltage-gated ion channels

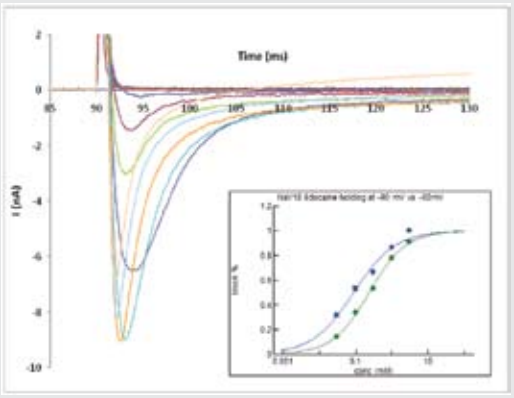
Voltage-gated ion channels are responsible for determining the shape, duration, and frequency of action potentials in excitable cells. Given this important physiological role they have been heavily pursued targets in drug discovery efforts. Voltage-gated channels also control important physiological functions in non-excitable cells, for example secretory epithelial cells.

**Key advantages for this application class:**

- Superior concentration clamp due to continuous perfusion
- High success rates due to ensemble recording
- Compound off-rate measurements
- Rleak and Rseries compensation available

**Representative applications:**

- Compound efficacy screening
- Compound profiling (IC50 determination)
- Mutant screening
- Mechanism of action studies
- Sodium (Nav) and potassium (Kv) channels




Sweeps in response to increasing voltage steps from -120mV to +20 mV were used to determine the current-voltage relationship for this Nav1.8 voltage gated sodium channel. The experiment demonstrated peak currents above 2nA for 90% of the ensembles tested, a high success rate for a target the usually suffers from low current amplitudes. Inset: IC50 shift for the blocker lidocane at different holding voltages demonstrates state-dependent pharmacology.

## hERG toxicology and safety screening

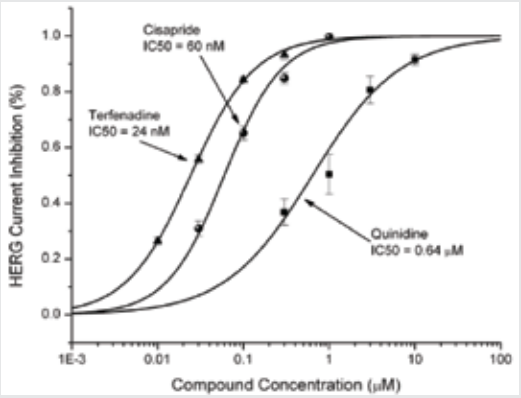
Due to the difficulty and expense of *in vivo* testing, the human ether-a-gogo related gene (hERG) channel continues to serve as an important surrogate indicator of potential cardiac liability. The ability to affordably assay early in the drug discovery process remains vitally important in the pharmaceutical industry. The IonFlux system is uniquely suited for hERG compound profiling work due to its ability to work with difficult compounds (low solubility / high lipophillicity) and important gains in throughput, success rate, and cost as compared to other assay types.

**Key advantages for this application class:**

- Superior concentration clamp due to continuous perfusion
- High success rates due to ensemble recording
- Temperature control

**Representative applications:**

- hERG safety profiling (IC50 studies)
- Compound screening for hERG liability
- hERG studies at physiological temperature




Dose response data (hERG current inhibited as a function of concentration) is shown for three different known hERG blocker compounds: Cisapride, Terfenadine and Quinidine. The hill function fit yields IC50 values that are in good agreement with literature values. Of particular importance are lipophillic compounds suchas terfenadine, which in this experiment was shown to have an IC50 value of 24nM.

## Temperature dependent effects

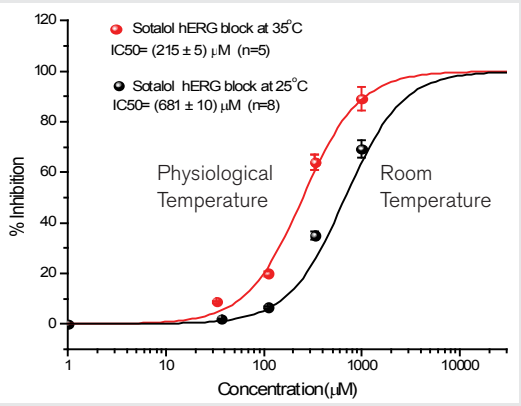
Temperature may affect both the kinetics of the ion channel as well as the pharmacological response to modulating compounds. This is rarely done in the early stages of the drug discovery process due to the difficulty of assaying at physiological temperature . The unique, microfluidic design enables high throughput experiments at physiological temperature.

**Key advantages for this application class:**

- Superior temperature control, no thermal shifts during solution change
- Increased consistency of response by maintaining consistent temperatures across different experiments
- Temperature can be elevated after the seal step, increasing success rates
- Fast temperature equilibration time of 30-60s

**Representative applications:**

- Safety pharmacology
- Sodium channel kinetics (NaV 1.1 - 1.8)
- Potassium channel kinetics (Kv1.1 - 11.1)
- Membrane porator activity




For safety pharmacology applications, it is important to determine the effect of temperature on the hERG activity of candidate compounds. For DL-sotalol, tested above, the IC50 shifts by a significant amount when experiments are conducted at physiological temperature (35˚C in this case) as compared to room temperature values. The shift observed on the IonFlux instrument fits existing literature values.

# A system that meets YOUR needs

The IonFlux System was designed with a keen understanding that every lab has its own unique needs. That's why the IonFlux comes in two distinct configurations to meet your specific requirements for throughput, automation, and budget.

## IonFlux HT System


- Highest throughput system available
- Lowest cost per data point
- Compatible with lab automation hardware
- Space saving footprint

The IonFlux HT System is the highest throughput automated patch clamp system available today. It has 64 patch clamp amplifiers which run in parallel to produce up to 8000 data points per day. Typical applications include drug screening, pharmacology, and cardiac safety profiling (hERG).

## IonFlux 16 System


- Ideal for ion channel research and assay development applications
- Automated patch performance for the cost of a manual patch clamp rig
- No patch clamp expertise required

The IonFlux 16 System is the world's first mutli-channel automated patch clamp system designed for the individual research lab. It has 16 patch clamp amplifiers which run in parallel. Typical applications include functional expression of ion channels, mutant screening, and drug profiling.

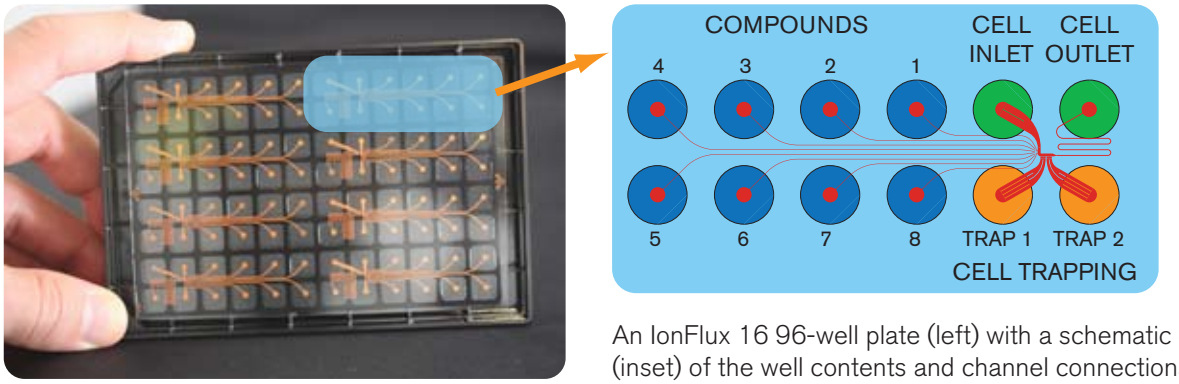
# IonFlux Plate Configurations

IonFlux Plates are consumable devices featuring Well Plate Microfluidic™ technology. There are different plate designs to accommodate a wide range of ion channel applications. They are integrated into SBS-standard well plate formats for convenience and compatibility with automated liquid handling instrumentation.

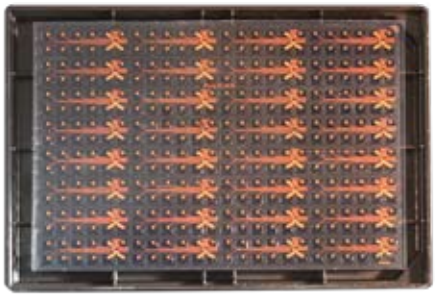
## Experimental Patterns

IonFlux microfluidic well plates contain an array of experimental patterns. Each pattern consists of 12 wells that are used to carry out an ion channel experiment. The wells are all connected to each other through microfluidic channels which run underneath the well plate. Within a pattern, two of the wells are used to control the cell trapping process, and two wells are used as an input and output for the cells. The remaining eight wells receive a unique compound or concentration series. All eight compounds can be applied to the ensemble of cells trapped in a particular experimental pattern.

IonFlux 16 Plates each contain 8 experimental patterns on a 96-well plate format, whereas IonFlux HT plates each contain 32 experimental patterns on a 384-well plate format.




An IonFlux 16 96-well plate (left) with a schematic depiction (inset) of the well contents and channel connections.



An IonFlux HT 384-well plate (left) showing 32 experimental patterns

| | IonFlux HT / 384-well | IonFlux 16 / 96-well |
|---|---|---|
| Plate format | SBS-standard<br>384 well plate, square well | SBS-standard<br>96 well plate, square well |
| Working volume of well | 100µL | 250µL |
| Number of experimental patterns | 32 | 8 |
| Number of compounds per pattern | 8 | 8 |
| Number of cell recordings per pattern | 2 | 2 |
| Number of cells trapped per recording | 20 | 20 |
| Compounds tested per plate | 256 | 64 |
| Total data points per plate | 512 | 128 |

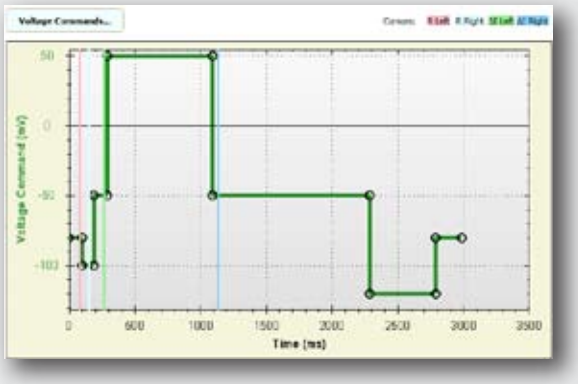
# Intuitive software control for maximum productivity

## IonFlux Software

The IonFlux Software makes it easy to setup, run, and analyze complex ion channel experiments. The simple graphical interface and intuitive workflow enables users to get started quickly and increase productivity.

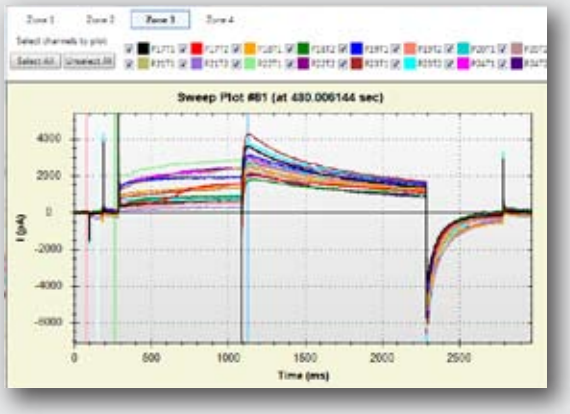
### Setup Module

The Setup Module allows quick configuration of plate layout and compound identification. The experimental sequence is created using a simple graphical interface. Voltage protocols and compound addition timings are both easily controlled.



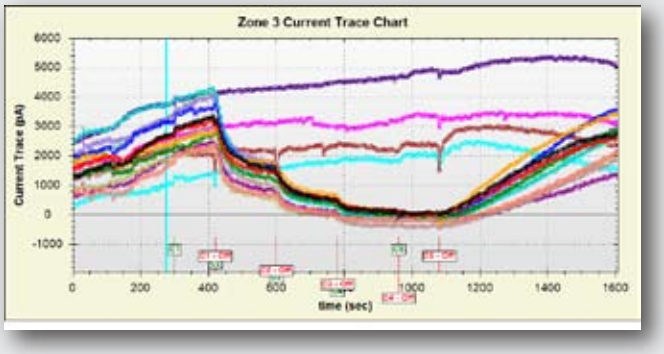

### Runtime Module

The Runtime Module enables convenient visualization of experiments. Sweep traces can be viewed in real time over the duration of the experiment. R-t and I-t charts are plotted for all recording channels.




### Data Analysis Module

The Data Analysis Module facilitates review and analysis of current characteristics and compound pharmacology. Data can easily be exported and analyzed in common database software applications.



# IonFlux Specifications

| | IonFlux HT System | IonFlux 16 System |
|---|---|---|
| **System performance** | | |
| Daily throughput | 8000 data points | 2000 data points |
| Number of simultaneous amplifier channels | 64 | 16 |
| Cell recording configuration | Ensemble recording | Ensemble recording |
| Compound application time | <100ms | <100ms |
| **Plate format** | | |
| Plate type | 384-well, SBS format | 96-well, SBS format |
| Total data points per plate | 512 | 128 |
| Unique compounds/concentrations per plate | 256 | 64 |
| Number of experimental patterns per plate | 32 | 8 |

| | System Specifications Common to IonFlux 16 and IonFlux HT |
|---|---|
| **Instrument configuration** | |
| Instrument dimensions | 20” (50 cm) X 20” (50cm) X 10” (25 cm) |
| Amplifier configuration | Sampling rate adjustable to 20 khz<br>Capacitance, leak, series resistance compensation |
| Temperature control | Ambient to 40°C |
| Cell recording format | 20 cells per ensemble recording, 2X redundancy |
| **Software** | |
| Operation modules | Protocol editor, run table, assay development mode, recording visualization, analysis, and data export |
| Operating system | Windows 7, XP, Vista |
| **External compatibility** | |
| Liquid handling automation | Compatible with most liquid handlers, SDK available |
| Multiplexing ability | Integrate up to 4 readers per liquid handler |

## Ordering Information:

**Systems**

| | |
|---|---|
| IonFlux 16 System (Instrument, Computer, Software) | P/N 950-0013 |
| IonFlux HT System (Instrument, Computer, Software) | P/N 950-0014 |
| Temperature Control Upgrade (add to IF-16 or IF-HT) | P/N 950-0018 |

**Well Plate Microfluidic Devices**

| | |
|---|---|
| IonFlux 16 Plates (8 plates per pack, 96-well format) | P/N 910-0044 |
| IonFlux HT Plates (8 plates per pack, 384-well format) | P/N 910-0045 |

## To request a quote or to place an order:

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FLUXION

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