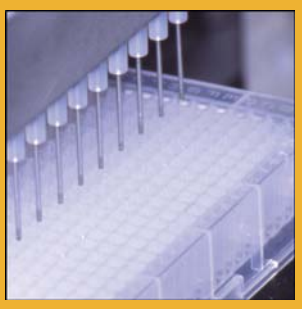


# The Ion Channel Reader Series

## Eliminating Bottlenecks in Ion Channel Research

Aurora Biomed's Ion Channel Readers are fully automated systems that use flame atomic absorption spectroscopy for detection of trace elements used for monitoring ion channel activity in cell-based flux assays. Ion channel activity within cells is determined by quantitating extracellular and intracellular concentrations of the trace elements. An example of a trace element used for monitoring ion flow in potassium channels is rubidium.

The instruments are fully programmable to allow for automatic dilution, calibration, washing, and analysis of samples in one convenient step. They are compatible with the existing automation and are currently used by many of the top pharmaceutical companies worldwide.

Ion channels play a pivotal role in a variety of cellular processes of the human body and have been associated with multiple diseases. Recent activities in the development of drugs that target ion channels have increased our understanding of channel modulators that offer significant therapeutic solutions to pathological conditions. High throughput screening of compound libraries has become the approach for identification of new compounds that target ion channels. Many technology platforms are currently utilized to address the bottlenecks within the drug discovery process.

In the past, the performances of most methods of ion channel analysis have typically fallen on the extremes of either accuracy or speed. The patch-clamp method is indisputably the golden standard for ion channel research as it offers the most accurate and high information data content, but it has a low throughput and requires the presence of a skilled electrophysiologists. Although fluorescent dye assays offer unsurpassed speed, they suffer from low accuracy, high background noise, high cost and limited capabilities for some ion channels (i.e. the hERG potassium channel). In an effort to address the current bottlenecks in ion channel drug discovery, Aurora Biomed has developed the ICR, which offers accuracy, precision and speed.

Aurora Biomed

## ICR 8000

- *Medium Throughput: Up to 5000 wells/day*
- *Single Channel:*
  *Measures 1 sample at a time*
- *Minimum sample volume: 50 µl*
- *Accommodation: 96/384-well microplates*
- *Footprint: H.67 cm X W.55 cm X D.37 cm*
- *Fuel Source: Acetylene / Compressed Air*
- *Options: Plate Stacker / Bar Code Reader*
- *Sensitivity: 0.05 ppm detection limit*
- *Precision: < 5% CV*

## ICR 12000

- *High Throughput: Up to 60,000 wells/day*
- *Multi-Channel:*
  *Measures 12 samples at a time*
- *Minimum sample volume: 20 µl*
- *Accommodation: 96/384-well microplates*
- *Footprint:*
  *H.135 cm(incl. plate stacker) X W.134cm X D.97cm*
- *Fuel Source: Natural Gas / Compressed Air*
- *Includes: Plate Stacker / Bar Code Reader*
- *Sensitivity: 0.05 ppm detection limit*
- *Precision: < 5% CV*

## ICR 8000

The ICR 8000 has medium throughput capabilities. It is a user-friendly system driven by a personalized software. Direct sample analysis allows for high levels of sensitivity; as little as 0.05 ppm $Rb^+$ can be detected. It has a 3-4 samples-per-minute throughput, and is compatible with 96 and 384-well format plates. Sample volumes can be as low as 50 µl, while still maintaining high detection levels.

## ICR 12000

The ICR 12000 has 12-fold higher throughput capabilities than the ICR 8000. This unit comes equipped with a plate stacker and bar code reader. It produces up to 60,000 data points per day and is considered Aurora Biomed's high throughput instrument.

## Applications

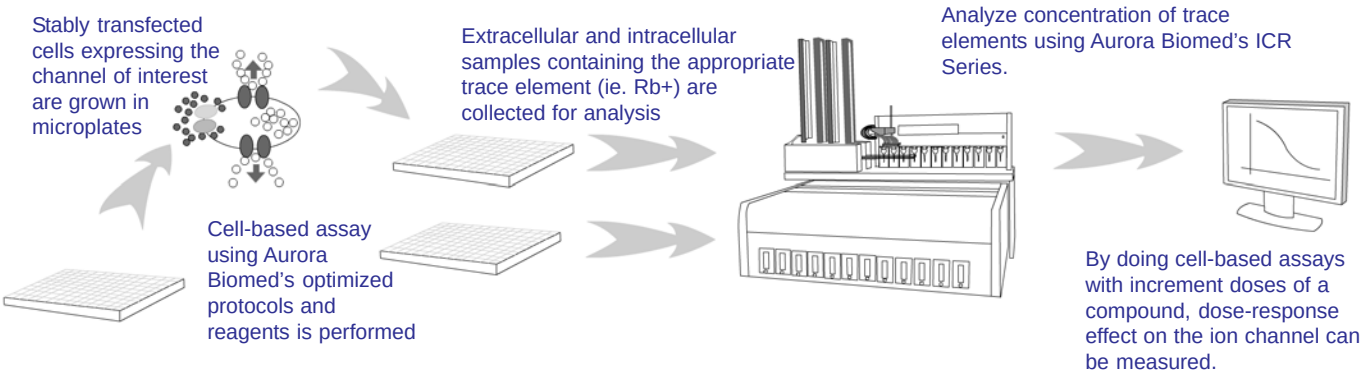
The ICR series is an accurate way to screen for novel compounds that modulate ion channel activity. Combined with flux assays, the system can be used for primary screening against ion channel targets or for secondary screening to assess drug safety.

The prevalent target in the safety screening is a hERG potassium channel. Many compounds have been pulled off the market because they interacted with the hERG channel and induced Long-QT Syndrome. Aurora Biomed's ICR is well-validated against this channel and is therefore one of the most advanced techniques used by pharmaceutical companies worldwide.

## Advantages

- *Reliable data recovery without sacrificing throughput. Screens can be carried out at 5,000 samples per day with the ICR 8000 or up to 60,000 samples per day with an ICR 12000 instrument.*
- *Circumvents the quenching effects found in fluorescence imaging detection.*
- *Eliminates the health risks, disposal requirements and work restrictions ($t_{1/2}$ = 18.6 days) associated with $^{86}Rb^+$ utilization.*
- *Minimizes human error with fully automated systems programmed for diluting, calibrating, and cleaning.*
- *Single point intergration instruments that are adaptable to existing automation.*
- *Microplate sampling capabilities eliminate the need for sample dilution, resulting in higher sensitivity.*
- *Successful in studying $K^+$ channel activity, but also applicable for other ion channels.*
- *A proven application for hERG $K^+$ channel assessments.*
- *Low running cost.*

## How is Aurora Biomed's ICR Series used in studying ion channels?




### Flux Assays

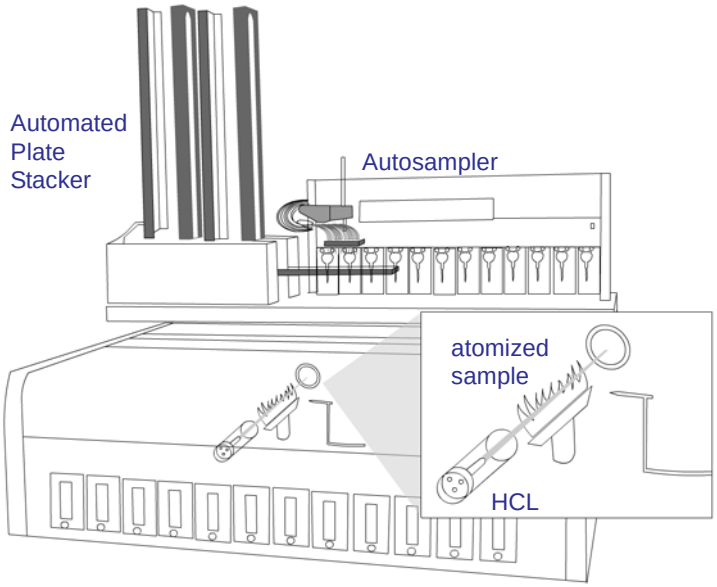
As described in literature, trace elements in flux assays are widely used to detect ion channel activity. These assays are designed to study ion channels by chemically initiating the opening of the channel and measuring the ionic flux of the trace ions. An example of such an ion is rubidium, which is used to study potassium channels because it is similar to potassium in size, charge, and channel-permeability. Furthermore, rubidium is not found in physiological systems; this eliminates interference from the background signals. Rubidium flux assays have been described for voltage-gated potassium channels including inward rectifiers, outward rectifiers, delayed rectifiers, calcium sensitive channels and ligand-gated channels. It is an assay that is well-documented in scientific literature and has been widely used for studying the potassium channel family[1].

Although the rubidium flux assay has been emphasized here, different trace elements can be used when studying other ion channels. Several candidates are currently being studied in order to develop new flux assays compatible with the ICR series.

Aurora Biomed is committed to the success of our existing clients and our new customers. We will regularly discuss the progress of our developments through newsletters and website updates.

## How does the ICR work?

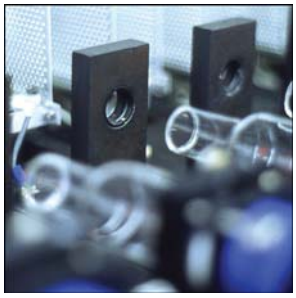
The ICR Series features a patent-pending process that couples microsampling to atomic absorption spectroscopy. The ICR 12000 that is shown in the diagram to the right functions as 12 individual units of the ICR 8000. Within each of these units, biological sample is delivered to the atomic absorption spectrometer via the autosampler arm. Once inside the atomic absorption spectrometer, the sample is atomized by a flame which then absorbs a specific wavelength of light emited by a hollow-cathode lamp (HCL). The absorbance of the sample is measured to determine the concentration of the element in the sample.

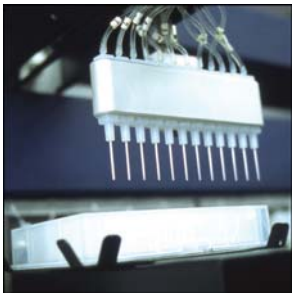


## Technology Comparison

| | Throughput | Information Content | Cost / data point | Ease of Use / Interpretation | Comments |
|---|---|---|---|---|---|
| **Electrophysiology** | *Low (<30 data points per day)* | *High (kinetics)* | *High* | *Requires highly skilled person.* | *Very labor intensive but high quality data* |
| **Automated Patch Clamp Electro-physiology** | *Medium (100 - 3000 data points per day)* | *High (kinetics)* | *High* | *Requires highly skilled person to interpret data* | *Not suitable for HTS. Data not always comparable to tradi-tional patch clamp. Low success rate.* |
| **Fluorescence** | *High* | *Low* | *Medium* | *Easy* | *Prone to quenching effects which lead to high false negatives and false positives.* |
| **Membrane Binding Assays** | *High* | *Low (non-functional Assay)* | *Low* | *Easy* | *Requires ligand avail-ability* |
| **Radioactive Ionic Flux Assays** | *Medium* | *Medium (end point analysis)* | *Medium* | *Easy, but hazardous* | *Has short half life and exposure concerns.* |
| **Non-Radioac-tive Flux Assays** | *High (60,000 data points per day)* | *Medium (end point analysis)* | *Low* | *Easy* | *Suitable for primary / secondary and safety screening across many ion channel classes.* |

Each technology has specific advantages but, when comparing throughput, cost per data point, overall ease of use and flexibility, it is clear that the ICR Series is the preferred choice for ion channel and drug safety screening.The Ion Channel Reader (ICR) series can reach the same experimental end points **with higher throughput** and **lower per data point costs!** Aurora pioneered the technology for high throughput screening using non-radioactive flux assays.This technology is currently being used by researchers for screening a variety of ion channel targets. Specifically, identification of hERG blockade for drug safety can now take place much farther up in high throughput screening pathway with the use of the ICR series. High through-put, flexibility, cost and data quality – that's the ICR series' advantage!



*References:*

1. *Terstappen, G.C. Functional Analysis of Native and Recombinant Ion Channels Using a High-Capacity Nonradioactive Rubidium Efflux Assay. (1999), Analytical Biochemistry 272; 149-155.*

2. *Deflarias, F.P. et al. Stable Expression of Human Kv1.3 Potassium Channel Resets the Resting Membrane Potential of Cultured Mammalian Cells. (1995), Receptors Channels 3(4); 273-81.*

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