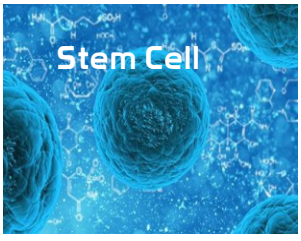
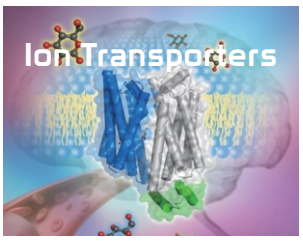
Aurora
ILLUMINATING SOLUTIONS

Ion Channel Reader Series

Eliminating Bottlenecks in Ion Channel & Ion Transporter Research





Ion Channel Reader Series

Aurora's Ion Channel Reader Series (ICR series) combine atomic absorption spectroscopy (AAS) with a patented microsampling technology to accurately measure ion movement in a cell-based assay format. This technology has been developed with the capability of measuring activity of voltage-gated and ligand-gated ion channels, co-transporters and pumps. It is considered an effective and high throughput solution to investigate a broad range of membrane proteins including electroneutral targets, to which conventional electrophysiology cannot be applied.

The ICR series detect ion movements across membrane proteins through quantifying intracellular and extracellular ion concentrations of interest using AAS. This is a technique that is independent of, and complementary to methods that rely on voltage manipulation. Since ion flux is a direct measure of channel activity, such assays are robust and less sensitive to disturbances. Data generated by the ICR Series are very consistent and predictive of drug potency.

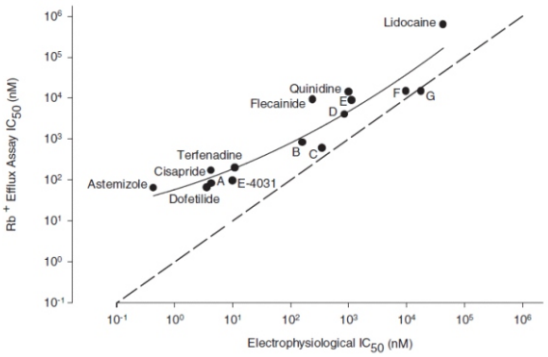
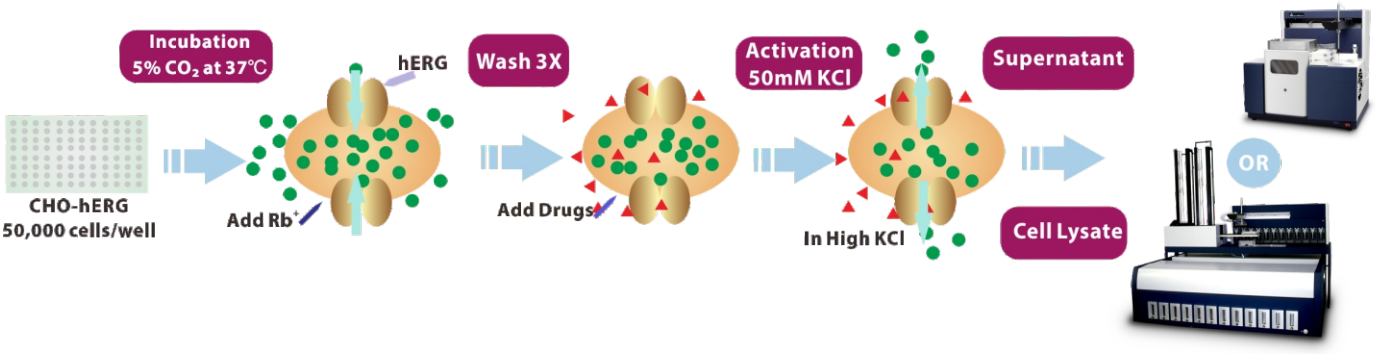


Figure 1. *The IC_{50} values obtained from 15 compounds using the Rb^+ flux method and manual patch clamp show a R^2 value of 0.83 (unbroken line). The broken line is for reference only.*

Table 1. *Consistent drug potency ranks have been established using the ICR Series and radioactive rubidium method.*

Test Compound	ICR 8000™ IC 50 (μM)	ICR 12000™ IC 50 (μM)	^{86}Rb IC 50(μM)	Same Rank order for all 3 assays
4636277	0.72	0.89	0.3	1
Bumetanide	1.16	1.17	1.5	2
993437	1.76	1.60	5.9	3
4653400	4.18	5.16	12.0	4

Principle of Ion Flux Assay



The procedure of setting up a flux assay is similar across all ion channel families. Cultured cells are first loaded with Rb^+ (or another tracer ion) and incubated overnight in a standard condition. This is followed by Rb^+ removal from the extracellular fluid using a wash buffer deprived of Rb^+. The compound of interest is then added into the wash buffer at a desirable concentration and incubated for an optimized time period. Activation of the ion channel under study then leads to Rb^+ efflux into the cell supernatant due to the established concentration gradient for this tracer ion. For voltage-gated channels this can be accomplished by adding a depolarizing buffer to the cells and for ligand-gated channels by adding the appropriate ligand. To measure the effect of potential channel modulators, both cell supernatant and lysate are collected with their tracer ion content measured by the ICR series. Ion efflux can be expressed as a ratio between extracellular and overall tracer ion content, thus eliminating potential well-to-well differences in cell densities and Rb^+ loading.

Dose Response Curves Generated by the ICR Series

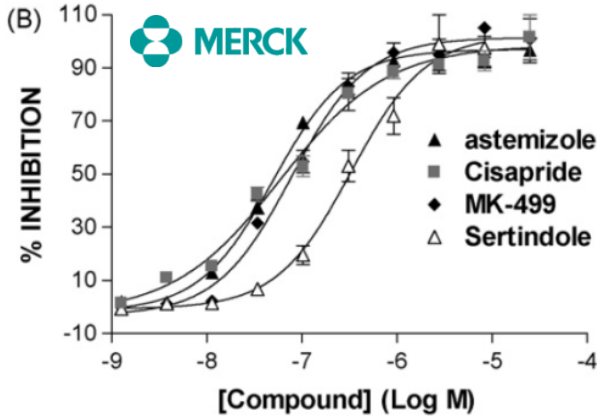
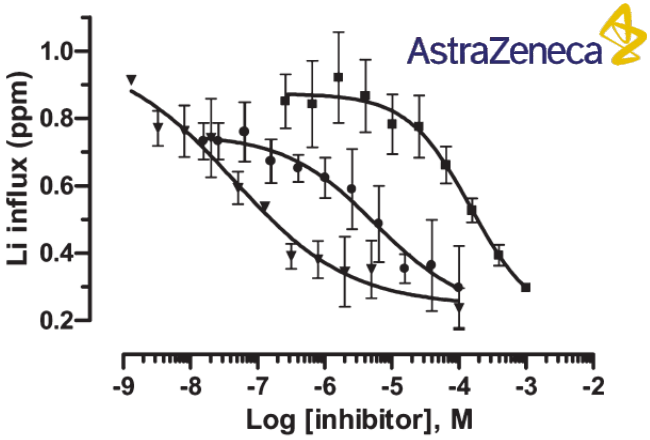


Figure 2. Rb^+ Flux Assay on hERG.
hERG antagonists astemizole, cisapride, MK-499 and sertindole displaying concentration-dependant channel inhibition effect in CHO-hERG cells.

Figure 3. Li^+ Flux Assay on Nav1.7
Nav1.7 inhibition curves for TTX (▼), tetracaine (●), and lidocaine (■)in Nav1.7 stably expressing HEK293 cells.



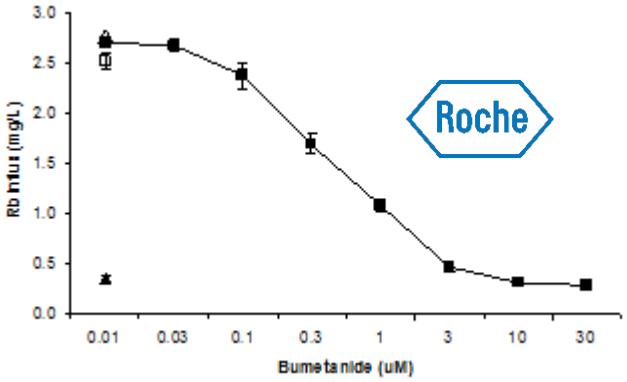


Figure 4. Rb^+ Flux Assay on NKCC1.
Concentration-dependant inhibitory effect of bumetanide on NKCC1 Rb^+ influx activated for 2 min (■), with positive control of 30μM bumetanide (▲), and negative controls with absence (Δ) and presence of 1 μM of digoxin (□).

Applicable Ion Channel & Transporter Targets

Using non-radioactive assay as a screening tool of membrane protein modulators is well-documented in scientific literature and has been widely used for studying the potassium channel family. It is developed to circumvent problems associated with the short-half life and high-energy emission of radioactive ^{86}Rb, while maintaining the information content and accuracy of the radioactive method. Rubidium is the most commonly used tracer ion to study potassium channels because of its similar physical properties to K^+, little natural presence in physiological systems, and ease to detect by AAS. The principle of the non-radioactive Rb assay can be easily applied to other membrane protein targets as well.

Tracer Ion	Applicable Targets
Rb^+	Potassium Channels/Transporters: hERG, KCNQ2, Kv1.1, Kv1.3, Kv1.4, Kv1.5, Kir6.2, B/SKCa, Slack, K_{ATP}, NKCC1, Na^+,K^+,-ATPase and more
Ag^+	Chloride Channels/Transporters: KCC2, TMEM16A, CFTR and more
Li^+	Sodium Channels: Nav1.2, Nav1.5, Nav1.7 and more
Ca^{2+}/Sr^{2+}	Calcium Channels: Cardiac L-type and more

Table 2. *The application of flux assay is not limited to studying potassium channel activities. Other tracer ions including Ag^+, Li^+, Ca^{2+} and potentially more can be used to screen against different targets in a flux assay format on the ICR series.*

Comparison Between Available Screening Technologies

There are several alternative methods widely available for assessment of ion channel activity. However, only the ICR series can deliver unparalleled speed, precision and reproducibility.

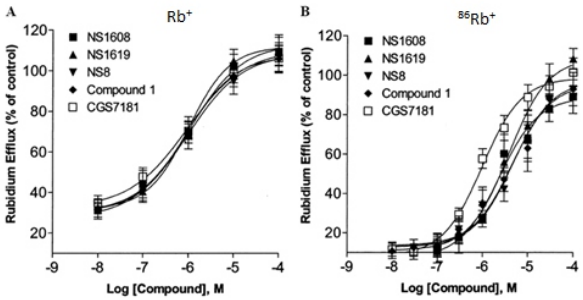
Method	Information Content	Throughput	Sensitivity	Accuracy	Comments
ICR 8000	Medium	Medium	High	Medium	Applicable to K^+, Na^+, Cl^-, Ca^{2+} channels and transporters
ICR 12000	Medium	High	High	Medium	Same as ICR 8000
Automated Electrophysiology	High	Medium	High	High	Not amenable to electro-neutral targets
Binding Assays	Low	High	Medium	Low	Requires radio-labeled probe specific for target
Radioactive Flux Assays	Medium	Medium	Medium	Medium	Short half-life and exposure concerns
Fluorescent Imaging	Low	High	Medium	Low	Prone to dye artifacts, high cost of consumables & high background noise

Reference Publications

The ICR Series are utilized as a tool to facilitate any ion channel and transporter research or screening where the measurement of ion movement provides meaningful insight into channel activity. The same principle of flux assay is amenable to studying more membrane protein targets than currently validated. Major pharmaceutical companies are especially in favour of the ICR given its automated workflow and high throughput. Academic institutions find that this technology provides reasonable data output at a low operating cost.

Functional Analysis of Large Conductance Ca^{2+}- Activated K^+ Channels

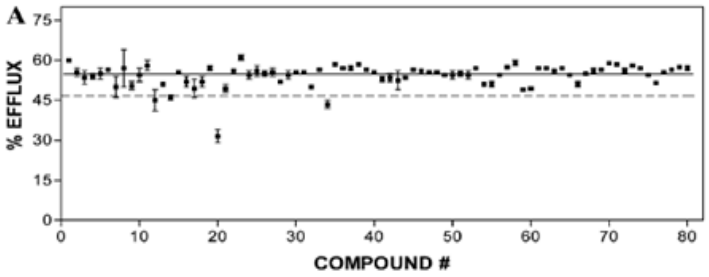
Abbott



The pharmacological profiles of BK(Ca) channels assessed by AAS (A) compare well with those obtained using the Rb^+ efflux assay (B).

Screening of KCNQ2 Potassium Channel Modulators

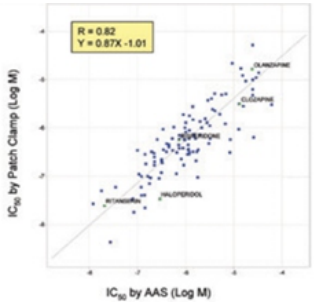
AstraZeneca



Testing 80 ion channel modulators for activity against KCNQ2. The solid line represents the average % efflux of all samples. The dashed line represents 20% inhibition of stimulated efflux.

Consistent Data Between Efflux Assay and Manual Patch Clamp Data on hERG Channels

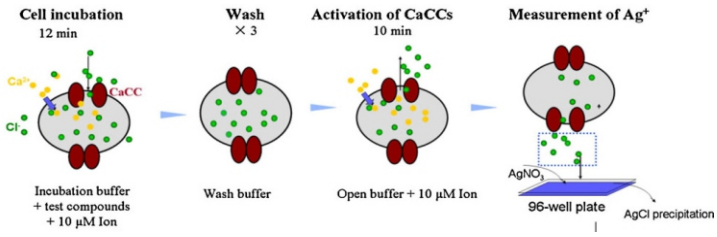
Lundbeck



Measurement of Rb^+efflux is a reliable and accurate method for predicting the potency of compounds (IC_{50} values) blocking hERG channels.

Using Ag^+ as a Tracer Ion to Study Modulators of Cl^- Channels

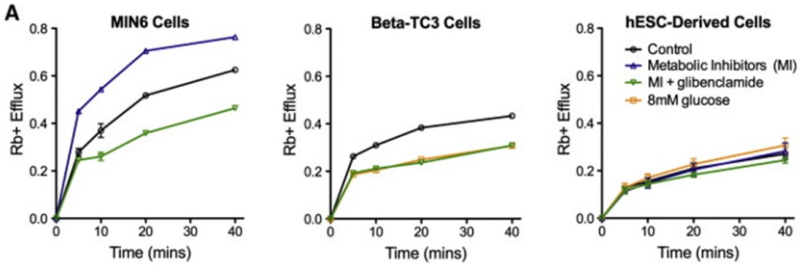
河北医科大学 HEBEI MEDICAL UNIVERSITY



AAS-based detection system for high throughput screening of CaCC modulators. Cl^- flux from CHO cells transfected with TMEM16A is assayed indirectly, by measuring excess Ag^+ ions in the supernatant of AgCl precipitate. The assay can be easily extended to study modulators of other Cl^- channel subtypes.

Measurement of Potassium Channel Activity in hESC-Derived Stem Cell Models

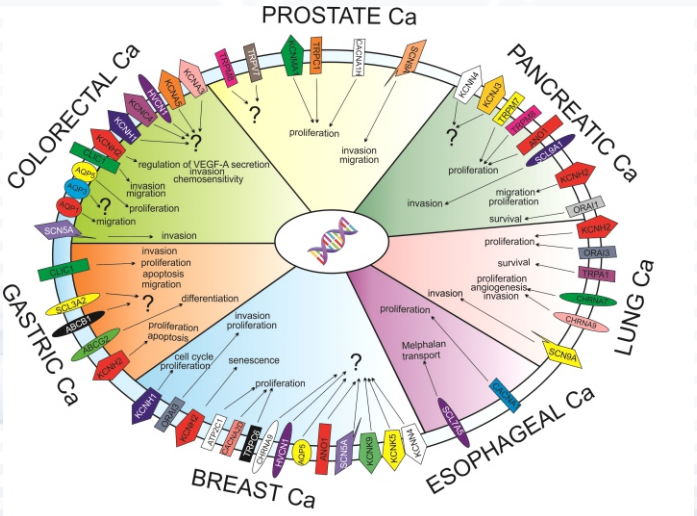
UBC THE UNIVERSITY OF BRITISH COLUMBIA



K_{ATP} channel activity was determined by measuring Rb^+ efflux over time. hESC-derived cells were not responsive to either K_{ATP} channel inhibitors (glibenclamide and glucose) or activators (metabolic inhibitors: oligomycin and 2-deoxy-D-glucose). In contrast, K_{ATP} channel activity in MIN6 β-cells was appropriately stimulated by metabolic inhibitors and inhibited by the addition of glibenclamide; both glibenclamide and glucose inhibited channel activity in another β-cell line, beta-TC3 cells.

Novel Perspectives of Ion Channel Research

Ion channels and transporters are a new class of membrane proteins aberrantly expressed in several types of human cancers. Besides regulating different aspects of cancer cell behavior, they can now represent novel cancer biomarkers. The University of Florence summarized how ion channels and transporters exert their functions in breast, prostate, lung, colorectal, esophageal, pancreatic and gastric cancers.



Specifications	ICR 8000™	ICR 12000™
Throughput	Up to 5000 wells/day	Up to 60000 wells/day
Minimum Sample Volume	50μL	20μL
Footprint (cm)	H67 × W55 × D37	H120 × W95 × D37
Sensitivity	0.05ppm detection limit	
Precision	< 5% CV	

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