Aurora

ILLUMINATING SOLUTIONS

ION CHANNEL READERS

ICR 8100 & ICR 12000

Label Free Technology for:

- Ion Channels
- Ion Transporters
- Pumps

ICR 8100

ICR 12000

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Ion Channel Readers

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 such as the patch clamp technique. Flux assays are robust and less sensitive to disturbances, and data generated by the ICR Series are very consistent and predictive of drug potency.

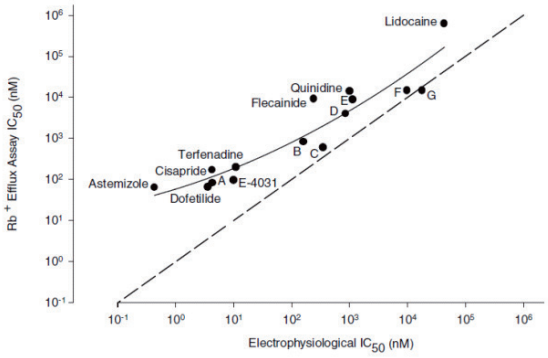
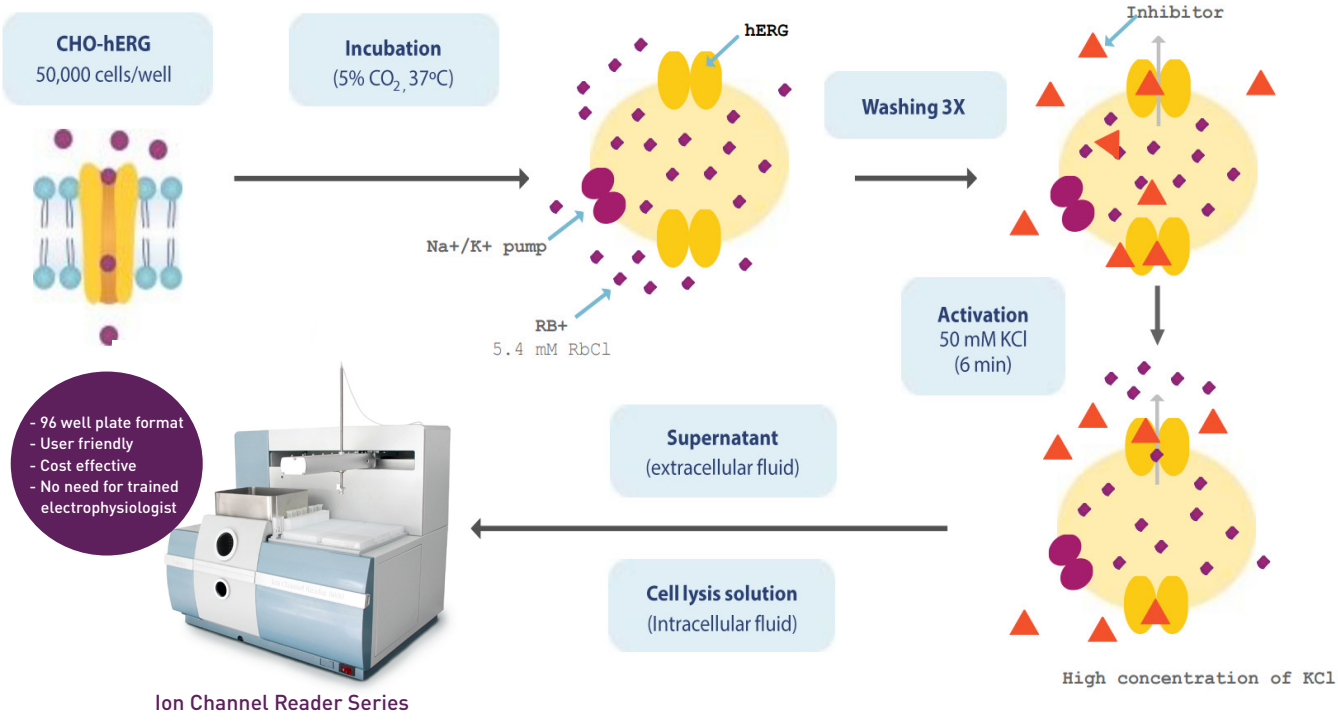


FIGURE 1. The hERG IC_{50} values obtained from 15 compounds using the Rb^+ flux method and manual patch clamp show a R^2 value of 0.83 (unbroken line). The broken line is for reference only. (Rezazadeh et al, 2004[6])

Principle of Nonradioactive Rubidium Efflux Assay Applied to hERG Channels



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

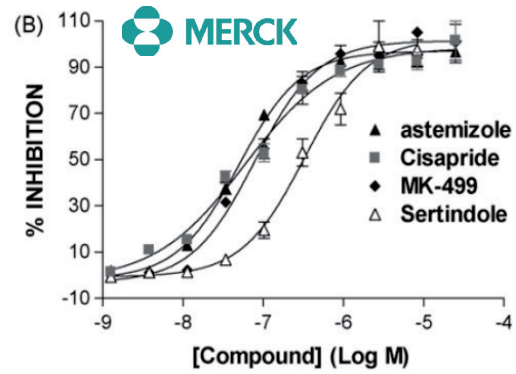


FIGURE 2. Rb^+ Flux Assay on hERG
hERG antagonists astemizole, cisapride, MK-499 and sertindole displaying concentration-dependant channel inhibition effect in CHO-hERG cells. (Karczewski et al, 2009[3])

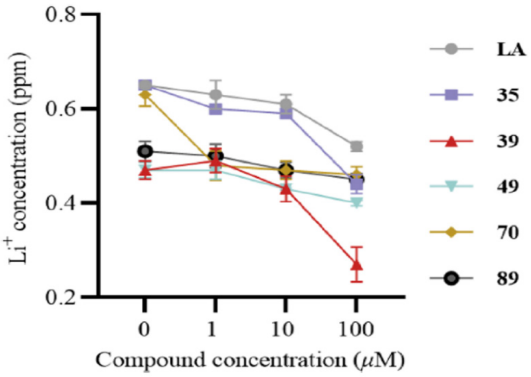


FIGURE 3. Li^+ Flux Assay on Nav1.7
Effects of test compounds on Li+ concentration in HEK-Nav1.7 cell lysates. (Li et al, 2022[12])

Test Compound (NKCC1 inhibitor)	ICR 8100 IC_{50} (μM)	ICR 12000 IC_{50} (μM)	^{86}Rb IC_{50}(μM)	Same Rank order for all 3 assays
4636277	0.720	0.890	0.30	1
Bumetanide	1.160	1.170	1.50	2
9934371	0.761	0.605	0.90	3
4653400	4.180	5.160	12.0	4

TABLE 1. ICR Series generate comparable data to the radioactive rubidium flux method without its concomitant safety and environmental hazards. (Yang et al, 2016[11])

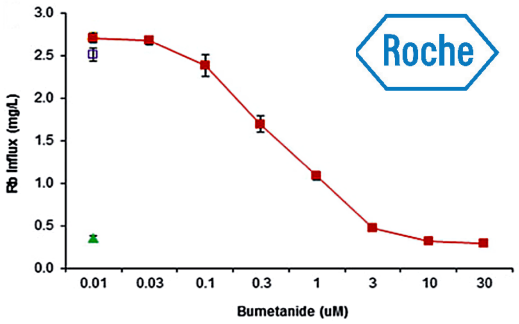


FIGURE 4. Rb^+ Flux Assay on NKCC1

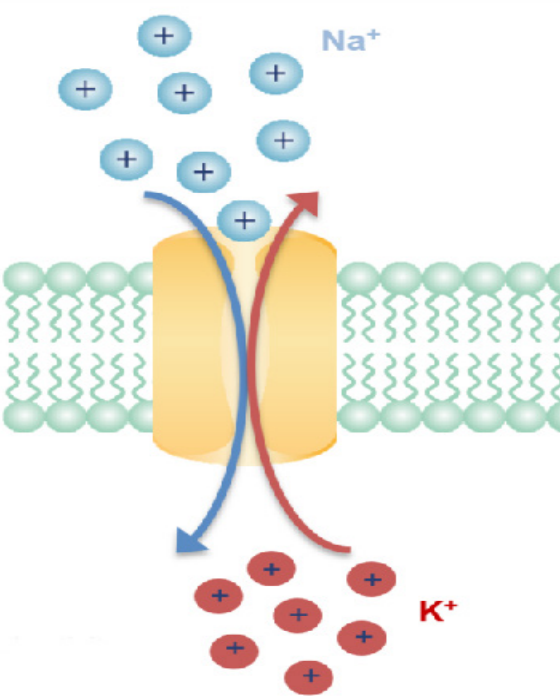
Concentration-dependant inhibitory effect of bumetanide on NKCC1 Rb^+ influx activated for 2 min (▲), with positive control of 30μM bumetanide (■), and negative controls with absence (△) and presence of 1 μM of digoxin (□). (Yang et al, 2016[11])

Applicable Ion Channel & Transporter Targets

Using non-radioactive assay as a screening tool for 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.

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.

Tracer Ion	Applicable Targets
Rb^+	Potassium Channels/Transporters: hERG, Kv7 channels, 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



Comparison Between Available Screening Technologies

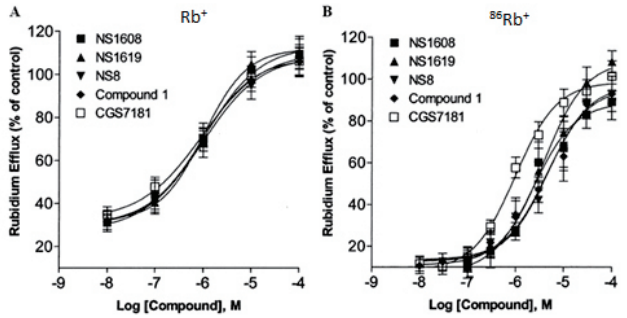
There are several alternative methods widely available for assessment of ion channel activity. However, only the ICR series can deliver unparalleled speed, precision and reproducibility.

Method	Information Content	Throughput	Sensitivity	Accuracy	Comments
ICR 8100	Medium	Medium	High	Medium	Applicable to K^+, Na^+, Cl^-, Ca^{2+} channels and transporters. Does not require trained electrophysiologist
ICR 12000	Medium	High	High	Medium	Same as ICR8000
Automated Electrophysiology	High	High	High	High	Not amenable to electro-neutral targets
Binding Assays	Low	High	Medium	Low	Requires radiolabeled probe specific for the targets
Radioactive Flux Assays	Medium	Medium	Medium	Medium	Short half-life and exposure concerns
Florescent Dyes	Low	High	Medium	Low	Induces false positives, high cost of consumables & high background noise

Reference Publications

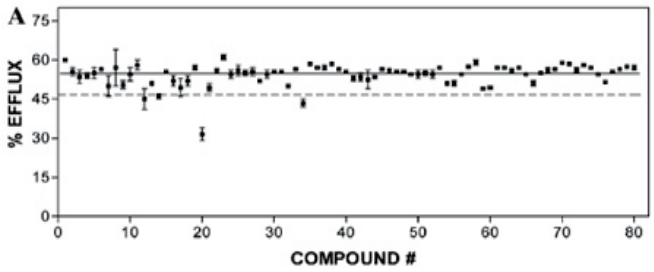
The ICR Series are utilized as a tool to facilitate any ion channel/ transporter research or screening where the measurement of ion movement provides meaningful insight into channel activity. The same principle of flux assay is amenable to studying more membrane protein targets than currently validated.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

FIGURE 5. Functional Analysis of Large Conductance Ca^{2+}- Activated K^+ Channels



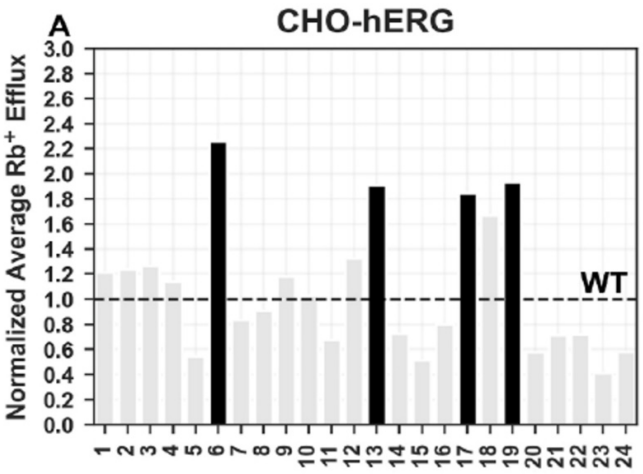
The pharmacological profiles of BK(Ca) channels assessed by AAS (A) compare well with those obtained using the Rb^+ efflux assay (B). (Parihar et al, 2003[1])

FIGURE 6. Screening of KCNQ2 Potassium Channel Modulators



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. (Scott et al, 2003[7])

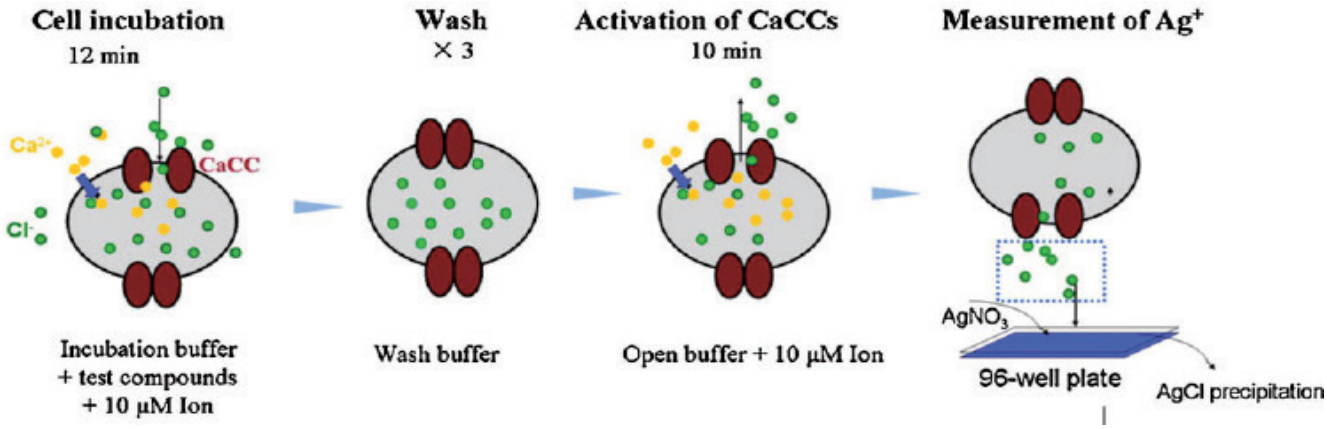
FIGURE 7. Rb^+ assay used for transfected hERG clone selection



Measurement of Rb^+ efflux is a method of choice for hERG transfected clone screening. (Montalbano et al, 2023[13])

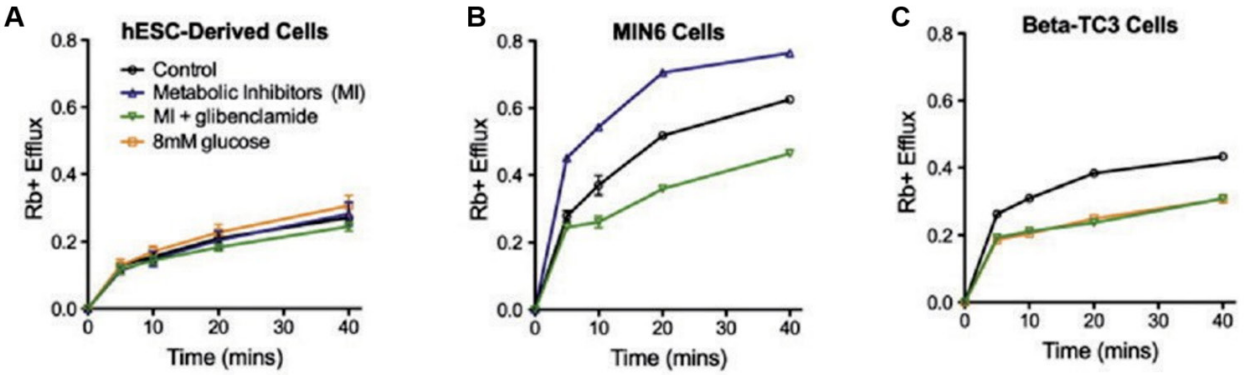
- 96 well plate format
- User friendly
- Cost effective
- No need for trained electrophysiologist

FIGURE 8. Using Ag^+ as a Tracer Ion to Study Modulators of Cl^- Channels



AAS-based detection system for high throughput screening of Ca activated chloride channel modulators (CaCC). 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 such as the cystic fibrosis transmembrane conductance regulator (CFTR). (Qi et al, 2014[5])

FIGURE 9. Measurement of Potassium Channel Activity in human embryonic stem cell (hESC)-Derived Models



K_{ATP} channel activity was determined by measuring Rb^+ efflux over time. A: 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). B: In contrast, K_{ATP} channel activity in MIN6 ß-cells was appropriately stimulated by metabolic inhibitors and inhibited by the addition of glibenclamide; C: both glibenclamide and glucose inhibited channel activity in another ß-cell line, beta-TC3 cells. (Bruina et al, 2013[14])

Reader Specifications

Specifications	ICR 8100	ICR 12000
Throughput	up to 5000 wells/day	up to 60000 well/day
Minimum Sample Volume	50 uL	20 uL
Footprint	H67 x W55 x D37	H120 x W 95 X D37
Sensitivity	0.05ppm dection limit	0.05ppm dection limit
Precision	<5% CV	<5% CV

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