

XRpro[®] Technology

Flexible, Label-Free Analysis of Transporters and Ion Channels

Applications

- Cell-based ion flux measurements
- Transporters and ion channels
- Electrogenic and non-electrogenic systems
- Experiments in simple buffers and complex media

XRpro[®] Technology: X-ray Fluorescence

XRpro technology leverages the unique capabilities of X-ray fluorescence (XRF) for analysis of plasma membrane transporters and ion channels. Direct spectroscopic measurements quantify elements in cell populations.

X-ray fluorescence is analogous to optical or UV fluorescence, but with individual atoms acting as fluorophores. With XRF, each element on the periodic table emits fluorescent X-rays with characteristic photon energies (Figure 1). A complete XRF spectrum is collected for each sample, allowing multiple target elements to be identified and measured simultaneously. XRF measurements are quantitative, with peak intensities directly proportional to target ion concentrations.

XRpro Targets

All elements with an atomic number of 13 (aluminum) or greater can be measured directly (Figure 2). Target analytes include biological monovalent ions (e.g., K^+), divalent ions (e.g., Ca^{2+}), transition metals (e.g., Zn^{2+}) and halogens (e.g., Cl^-). Surrogate ions such as Rb^+ and Sr^{2+} may also be used to improve precision.

Advantages of XRpro Technology

- Label-free - No dyes or radiolabels
- Analysis in complex matrices including 100% serum
- Broad range of targets - channels, transporters, cations, anions, and transition metals
- Simultaneous analysis of multiple target ions
- Linear conversion of signal to element concentration over 4+ orders of magnitude

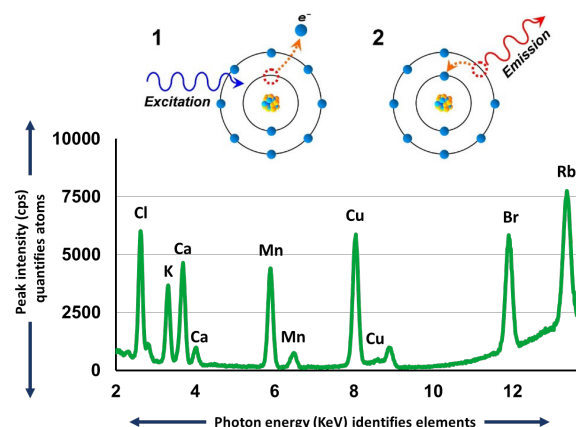


Figure 1: X-ray Fluorescence. (Top) 1. An excitation X-ray photon ejects an inner-shell electron from a target atom. 2. An electronic transition to fill the resulting hole emits a fluorescent X-ray. (Bottom) An example X-ray fluorescence spectrum. Each element generates unique peaks. Peak intensities are directly proportional to concentration.

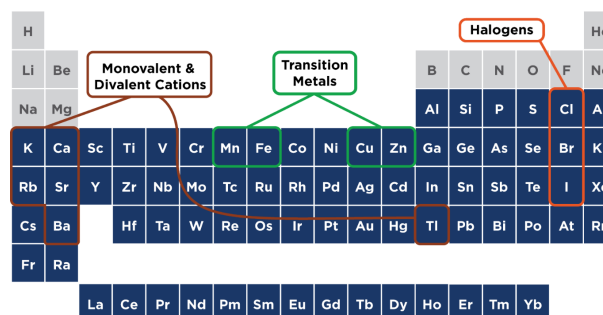
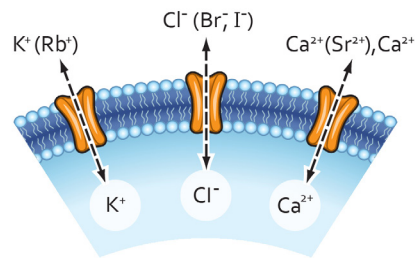


Figure 2: XRpro technology can directly measure all element with an atomic number greater than 13 (elements shown in dark blue).

Cell-based XRpro Assays

XRpro provides a straight-forward means to analyze ion flux in cell populations, targeting a broad range of channels and transporters including potassium, calcium, and chloride channels as well as zinc and phosphate transporters. Cell-based assay protocols use standard cell biology and buffers with cells grown in 96- or 384-well plates.



Phosphate Transporters

XRpro provides direct high-throughput analysis of both cation and anion transporters. Ions can be measured directly or using tracer ions such as Rb⁺, Sr²⁺ or arsenate to avoid background from endogenous K⁺, Ca²⁺ or phosphate.

- Activity measurements for Na-Pi family transporters (SLC34A, SLC20A)
- Overexpressed or endogenous transporters
- Arsenate tracer ion eliminates phosphate background
- Stopped kinetics allow V_{max} and K_M determinations

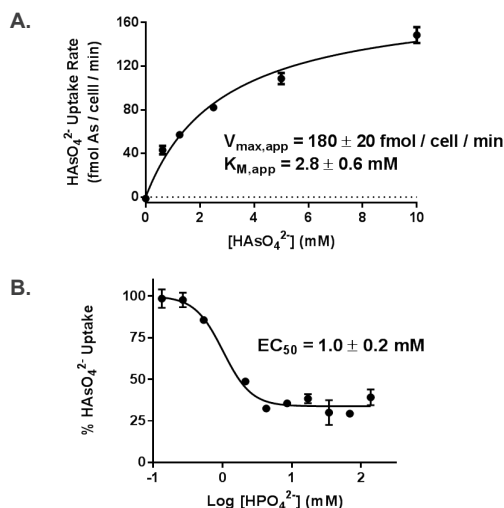


Figure 3: A. Endogenous phosphate transporter activity in HEK-293 cells measured following uptake of arsenate ions. B. Phosphate inhibits arsenate uptake, demonstrating that the two ions share a common transporter.

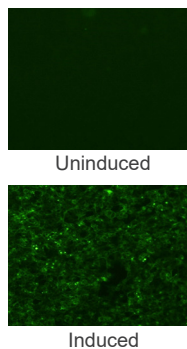
Zn²⁺ Transporters

Zn²⁺ plays a central role in numerous biological processes and disease, including diabetes. XRpro, combined with Icagen's established capabilities in custom cell line generation, are advancing Zn²⁺ transporter analysis.

- Direct measurements of Zn²⁺ flux
- Analysis of Znt1 Zn²⁺ efflux activity
- Applicable to other Zn²⁺ and transition metal transporters

Znt1 (SLC30A1) Zn²⁺ Efflux Measurement

A. Znt1-GFP Localization



B. Znt1 Zn²⁺ Efflux

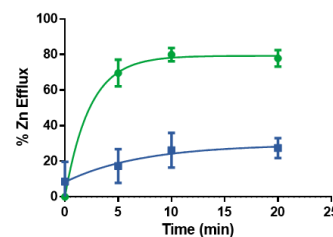


Figure 4: A. Fluorescence micrograph showing plasma membrane localization of GFP-tagged Znt1 in a HEK-293 based cell line. B. XRpro measurements demonstrate increased Zn²⁺ efflux in induced (green) vs. uninduced cells (blue).

For more information about working with Icagen to accelerate your ion channel and transporter research using XRpro[®] technology please contact us at: info@icagen.com

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