Thallium flux assay adaptation for multi-instrument compatibility

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Abstract

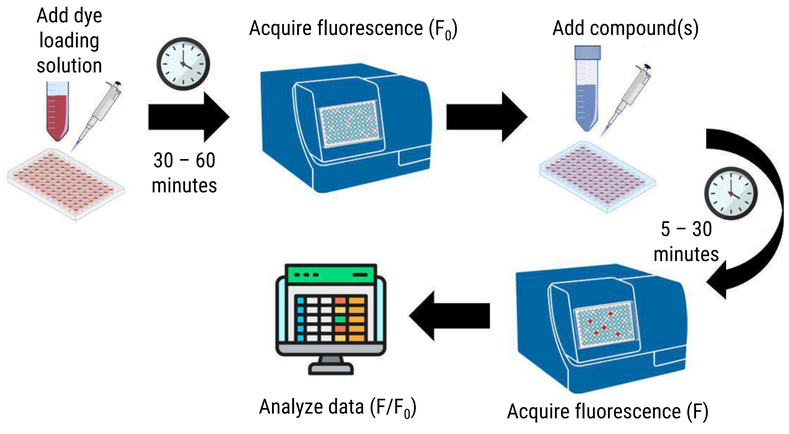
Over the past 15+ years, fluorescence-based measures of Tl^+ flux have brought about the discovery of small-molecule modulators of a host of ion channels, transporters, GPCRs and other targets of interest for both drug discovery and basic research.[1,2] Here, we introduce ION's **Brilliant Thallium *Snapshot*** Assay Kit, which provides a brand new way to conduct thallium flux assays. **Brilliant Thallium *Snapshot*** is designed for multi-well plate-based, high-throughput measurements of Tl^+ flux through K^+, Na^+, non-selective cation channels, and some Na^+ or K^+ transporters. Unlike traditional thallium flux assays, our patent-pending *Snapshot* assay format generates a **long-lasting signal** so you can easily detect and quantify changes in ion channel or transporter activity on most fluorescence-capable instruments. So whether you have a FLIPR® (HTS), a fluorescence microscope, a standard fluorescence plate reader, a high-content imager (HCS), or a flow cytometer - you can run functional screens on viable cells using thallium flux, enabling entirely new possibilities.

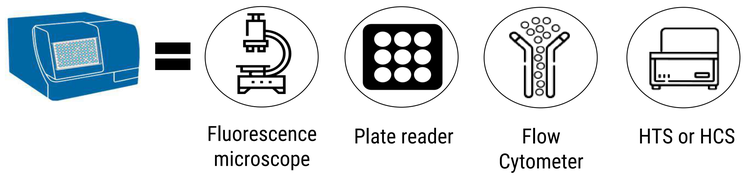
Instrument compatibility is demonstrated using a CHO GIRK1/2 (CHO G12) expressing cell line. GIRK channels are modulated using known activators, ML297[3] or VU0466551 (VU551).[4] Comparable EC_{50} values are obtained using data acquired from three commonly available instruments - a fluorescence microscope, standard fluorescence plate reader, and Flexstation®. Thallium flux paired with microscopy enables monitoring of specific cells within a diverse population - an ideal solution for measuring cell-specific ion channel activity in complex co-culture systems. Other applications of this technology could include clonal selection using FACS and identifying target expressing cells within dissociated tissue samples.

Methods

A simple workflow designed to accommodate a variety of instruments, automation, and users at all skill levels.

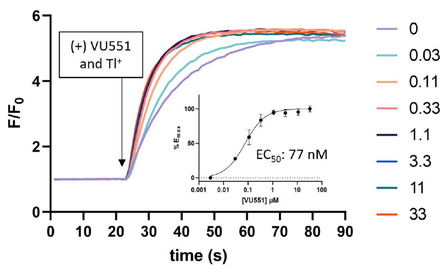
1) Prepare cell containing microplate
2) Remove medium (if possible)
3) Load cells with **dye loading solution** (contains Thallos AM and Tl^+), and incubate for 30 – 60 minutes
4) Acquire initial fluorescence readings (F_0) or images using appropriate filters (Ex/Em: 485/525 nm)
5) Add compound(s) of interest, and incubate for 5 – 30 minutes
6) Acquire fluorescence readings or images





Results

Figure 1. Traditional thallium flux assay. Rapid Tl^+ influx kinetics and fast equilibration limit the signal detection window. Inset) VU551 dose response curve with reported EC_{50} = 77 nM. Denoted concentrations are in µM.



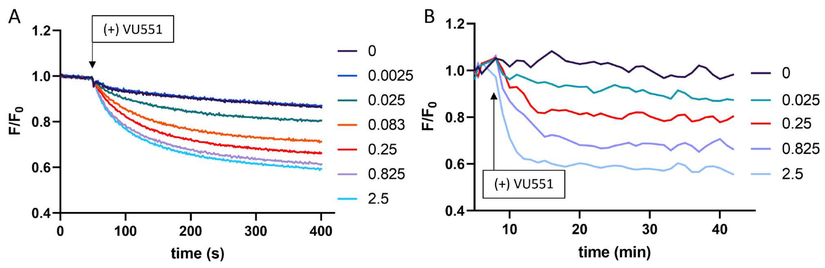


Figure 2. Kinetic fluorescence profiles of CHO GIRK1/2 (G12) cells using Brilliant Thallium Snapshot. A) Cells are pre-loaded with Thallos AM and Tl^+ prior to adding the GIRK activator, VU551. Kinetic data acquired using a Flexstation®. Efflux of Tl^+ upon GIRK channel activation yields a decrease in fluorescence. B) Changes in signal are sustained for >40 minutes as demonstrated using a standard fluorescence plate reader (Cytation 5). Reads were acquired at 1 min intervals. Denoted VU551 concentrations are in µM.

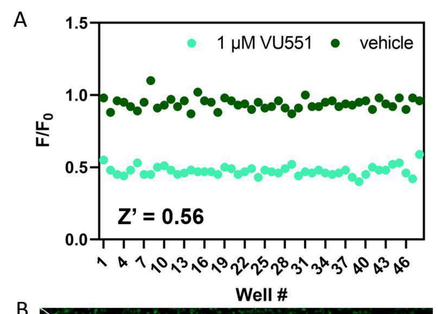


Figure 3. Brilliant Thallium Snapshot assays on a plate reader and microscope. A) Checkerboard layout of a 96-well plate was used to calculate a Z'. Vehicle and activator (1 µM VU551) treated wells are easily distinguished 20 minutes after compound addition (Z' = 0.56). B) Representative fluorescence images of vehicle- (left) and VU551-treated (right) CHO G12 cells demonstrating a 43% reduction in mean cellular fluorescence. Images acquired 20 min after VU551 addition using fluorescein filters and a 4X objective.

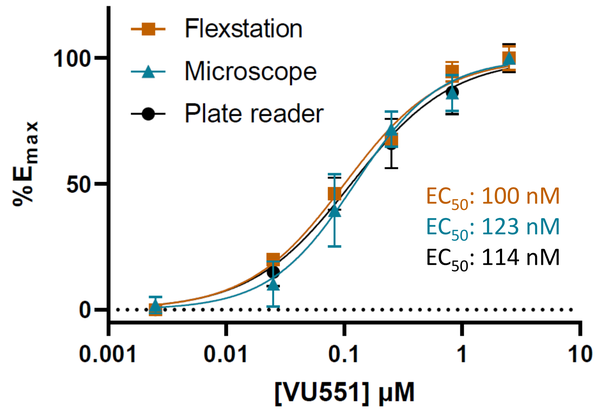


Figure 4. Dose-response curves of CHO G12 cells to VU551 measured using several instruments. When using Brilliant Thallium Snapshot Assay, comparable EC_{50} values are obtained on various fluorescence-capable instruments. All values are similar to data acquired using ION's Brilliant Thallium Flex kit (EC_{50} = 77 nM) and published literature (EC_{50} = 75 nM).

Results

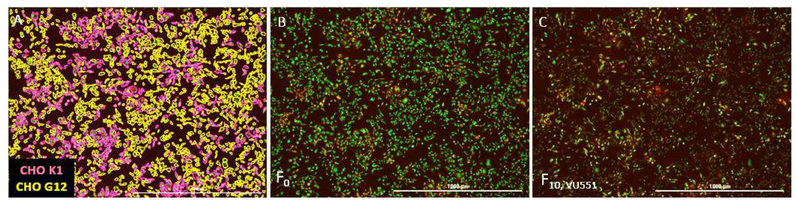


Figure 5. Imaging-based GIRK channel activity assays in a co-culture system. A) CHO K1 (pink) cells were stained with CytoTracker Red prior to plating in the same wells as CHO cells overexpressing GIRK1/2 (CHO G12 - yellow). CytoTracker Red fluorescence was used to identify cell type. B) Overlay of GFP and TRITC images acquired before the addition of VU551 (F_0). C) Overlay of GFP and TRITC images acquired 10 min after the addition of VU551 (F_{10}). A significant decrease in fluorescence was observed only in CHO G12 cells in the presence of VU551. Images (A - C) display the same field of view.

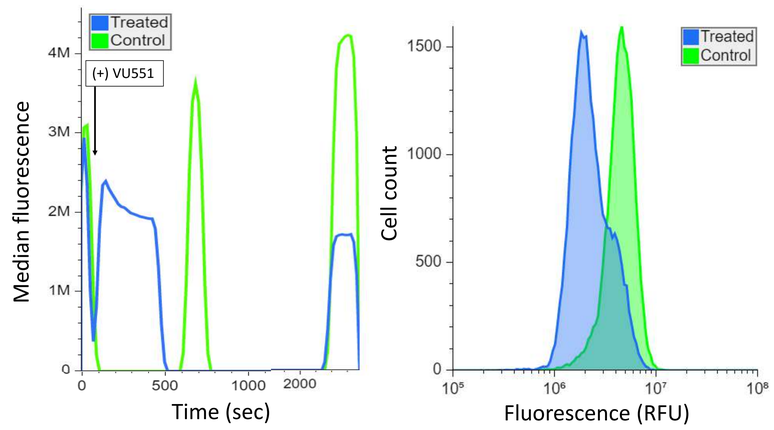


Figure 6. Flow cytometry-based GIRK channel assays. Left) Median fluorescence of separate cell populations acquired before and at reported timepoints after the addition of HHBSS or 3 µM VU551. Right) Histograms acquired 50 minutes post-treatment reveal a significant drop in cellular fluorescence in the group treated with a GIRK activator (1 µM VU551) relative to a control (HHBSS).

Conclusions

ION's **Brilliant Thallium *Snapshot*** assay delivers:

- **Sustained signal amplitude**
- **Increased accessibility**
- **Cell-specific readouts**
- **Greater convenience**
- **Reduced interference from background flux**

For additional information, visit product page here:

References

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3. Kaufmann K, Romaine I, Days E, et al. ML297 (VU0456810), the first potent and selective activator of the GIRK potassium channel, displays antiepileptic properties in mice. *ACS Chem Neurosci*. 2013.
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