Adaptation of a Cell-based Kinetic Potassium Channel Assay to an Endpoint Assay on Common Plate Readers and Microscopes

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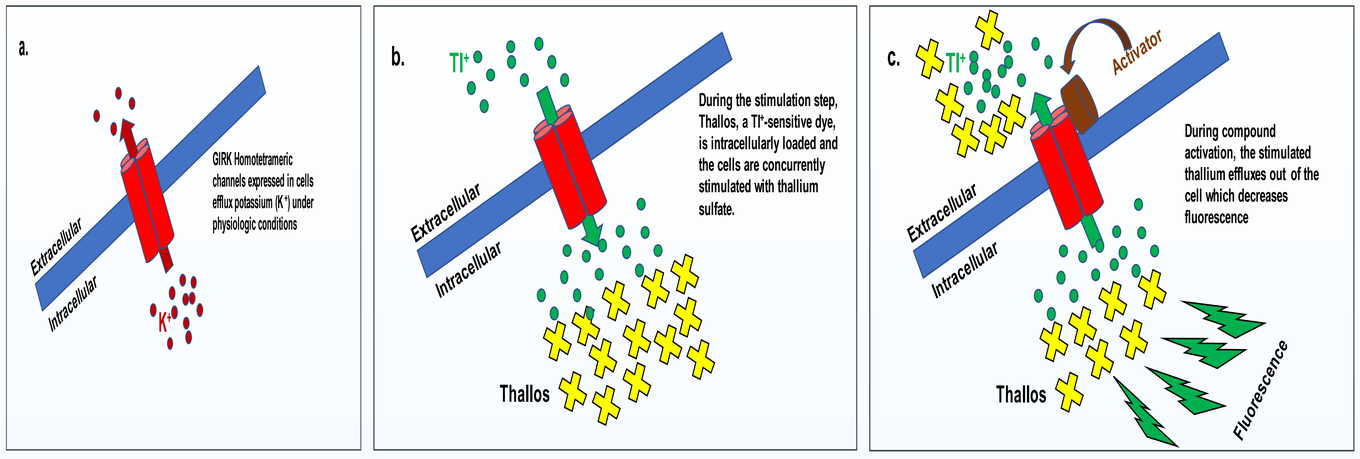
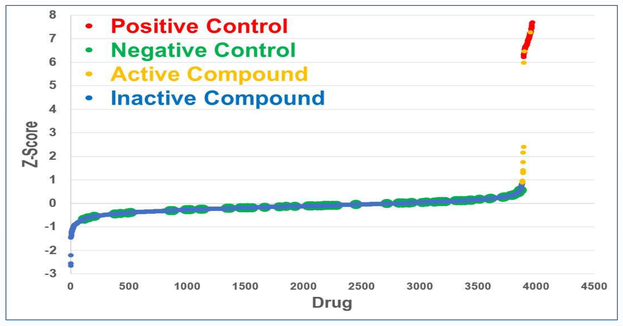
Abstract

Fluorescence-based potassium channel assays are typically run on expensive, hard to obtain, FLIPR based kinetic plate readers that are uncommon in most laboratories. Here we describe the use of the Brilliant Thallium Snapshot potassium channel assay from ION Biosciences that can be used across multiple plate reader platforms to identify modulators of potassium channels. For this work, we have taken the kinetic mode FLIPR based protocol and adapted it to be utilized on endpoint plate readers, such as the BMG Labtech Pherastar, to find activators of GIRK channels in CHO cells. We demonstrate that both plate readers are functionally competent at obtaining excellent Z's which makes them capable of finding corollary hits from the Sigma LOPAC 1,280 screening collection. Importantly this assay is also validated using the High Content Reader Thermo Cell Insight with the same Snapshot technology. The compendium of these results shows the flexibility, accessibility, and functionality of the channel assay readouts on more common plate readers.

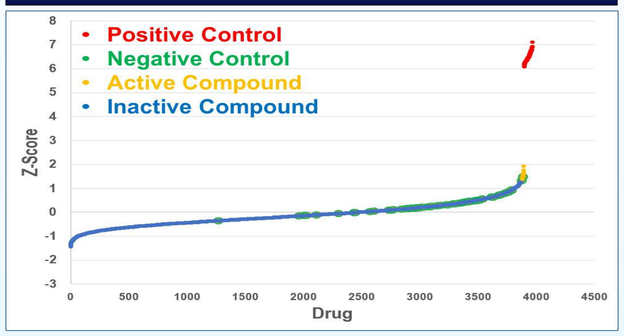
GIRK Channel Activation Experimental Design



LOPAC Pilot Z Scores (FLIPR)



Z Scores (Pherastar)

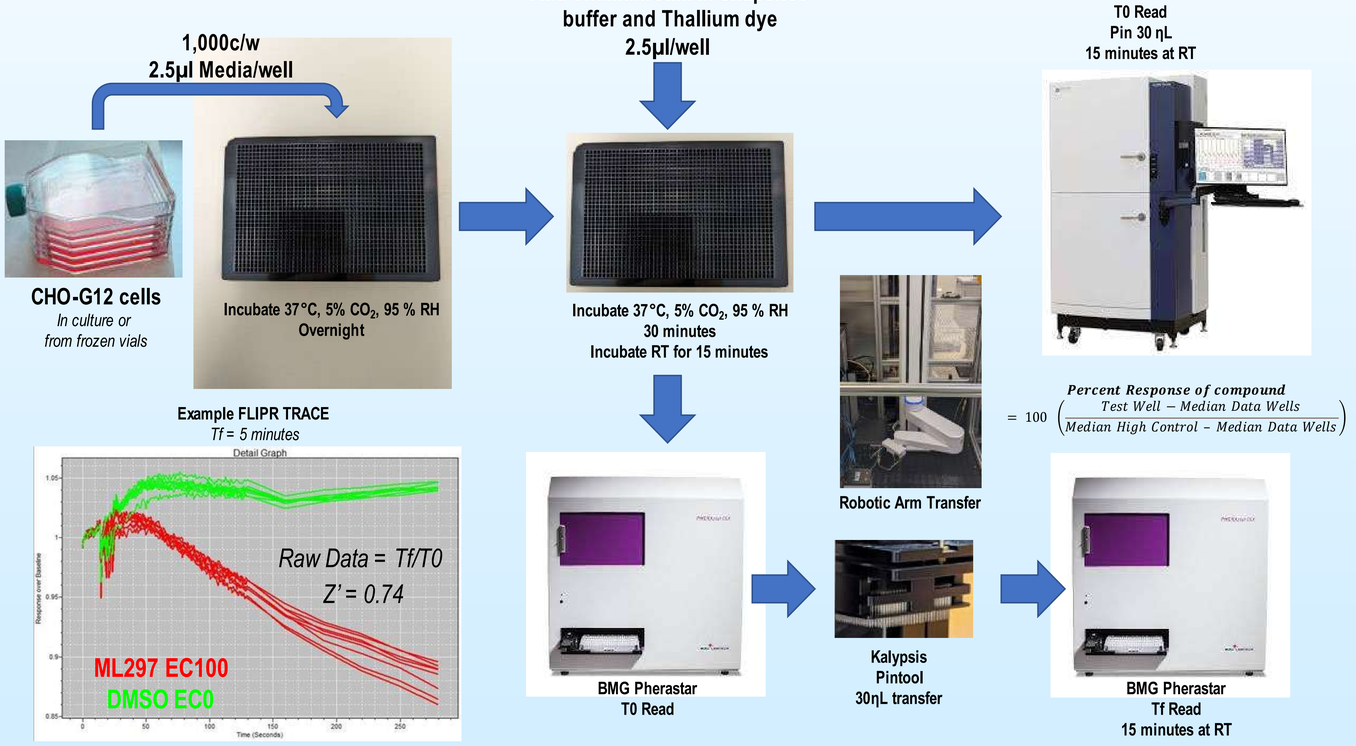


Brilliant Thallium Snapshot Dye Protocol

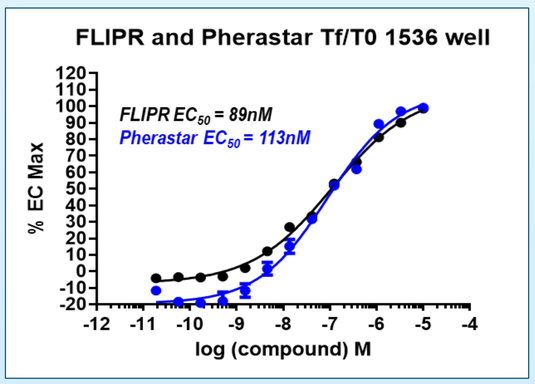
Reagent 10ml Final	Volume (µl)
Brilliant Thallium Indicator Solution	20
DySolv	20
10X Thallium Snapshot Assay Buffer	1000
TRS	200
Probenecid	200
50mM Thallium Sulfate Solution	50
In water up to	10ml

Note: 1X Solution of DySolv, Assay buffer, and TRS was provided by Ion Biosciences for this project

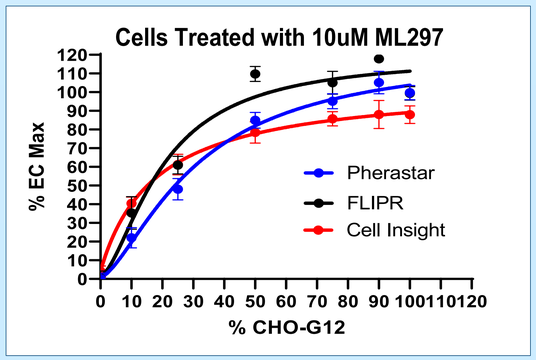
HTS Protocol



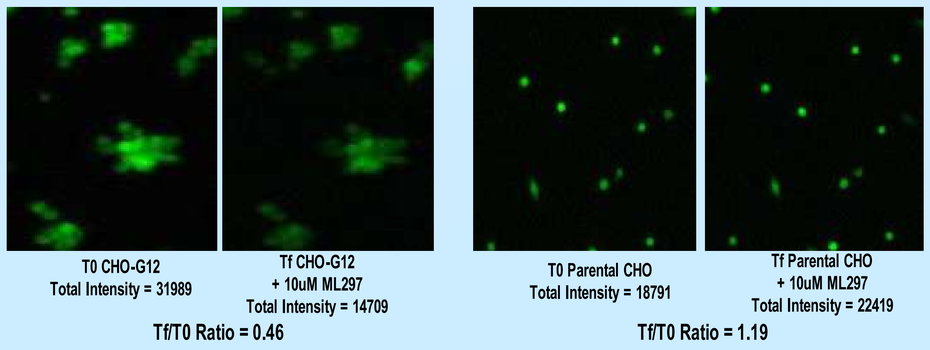
ML297 Positive CRC



CHO-G12 spiked with Parental CHO



Cell Insight Images (5X Magnification) Active vs Non-Active



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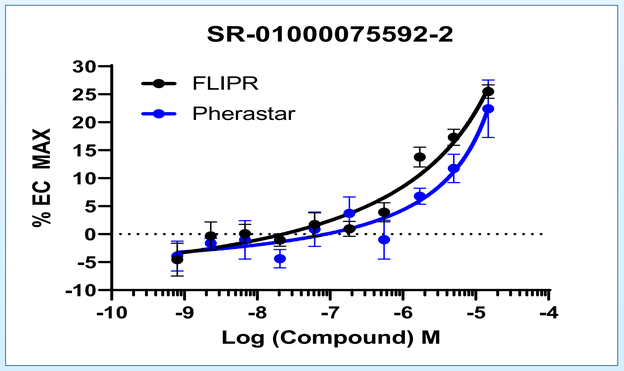
ACTIVE COMPOUND



Platform	Plates Tested	Compounds Tested (3X)	Screening Concentration	Z'	S:B	Hit Cutoff	# Hits	Hit Rate
FLIPR	3	1,275	6µM	0.75±0.03	1.20±0.02	14.17	4	0.31%
Pherastar	3	1,275	6µM	0.74±0.03	1.52±0.02	21.99	2	0.16%

Conclusions

- Brilliant Thallium Snapshot Technology from Ion Biosciences was validated in 3 different plate reader formats:
  - Molecular Devices FLIPR Kinetic Assay
  - BMG Pherastar Endpoint Assay
  - PerkinElmer Cell Insight High Content Assay
- ML297 potency is consistent in all reader formats
- SIGMA LOPAC 1,280 library was successfully tested using the FLIPR and Pherastar
  - 1 Compound showed some activity that correlated with the 2 readers (low activity compound)
- Spiking in parental CHO cells (negative cells) showed a cell dependent response in all 3 readers when treated with 10uM ML297
  - Images illustrate the difference between activated and non activated cells
- This effort illustrates the flexibility and adaptability of the Snapshot Thallium Flux assays towards conventional plate readers and beyond