A Novel Homogenous Potassium Ion Channel Assay for High-Throughput Screening

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OVERVIEW

Ion channels are a class of membrane proteins that mediate the movement of charged ions across the cell membrane. Potassium channels constitute the largest and most diverse group of ion channels, and they are expressed in virtually all cell types. Potassium channels are responsible for a variety of cellular functions including the maintenance and regulation of membrane potential, secretion of salt, hormone, and neurotransmitters. Not surprisingly, the dysfunction of potassium channels has been associated with many human diseases and off-target drug effects on potassium channels have been linked to cardiac toxicity. Due to their crucial physiological functions and their implication in drug-induced toxicity, potassium channels are heavily investigated by the pharmaceutical industry. Furthermore, cell-based functional assays have increasingly been used because they yield more physiologically-relevant results. However, challenges exist in measuring K^+ ion channel activities in a high throughput format. A common method employed is to use a potassium surrogate, thallium, coupled with thallium-sensitive fluorescent dyes. We have developed a new reagent based on this technology, the FLIPR® Potassium Assay Kit, and demonstrate its use for analyzing potassium ion channel activities on a FLIPR Tetra System. This reagent kit provides a homogeneous, fast, simple and reliable fluorescence-based high-throughput assay for potassium channel activity. Data collected with different types of potassium channels, such as Kv1.3 and hERG, as well as the comparison data against other existing technologies are presented.

ASSAY PRINCIPLE

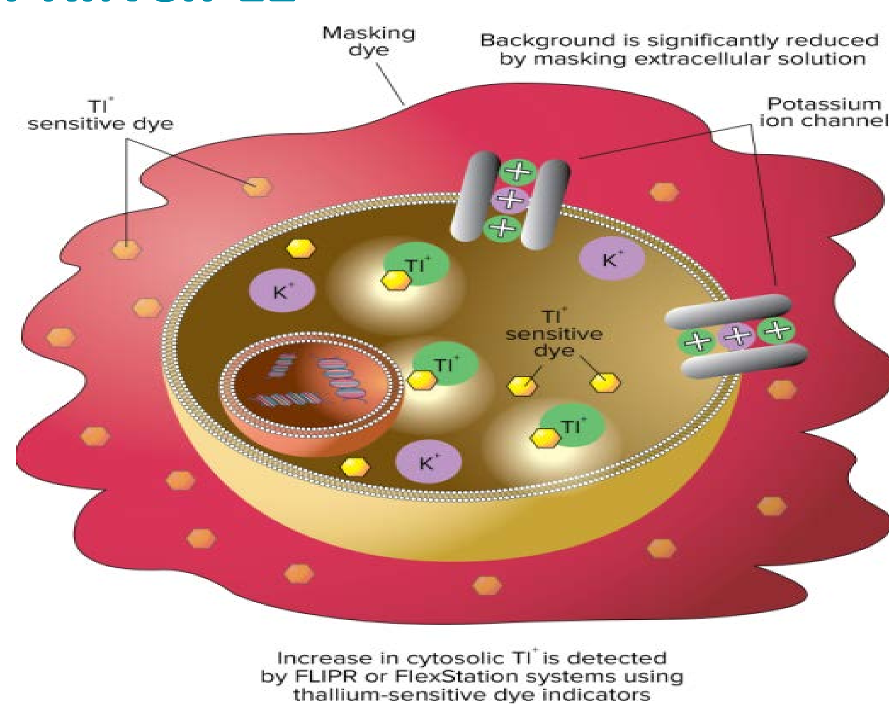


Figure 1. Cartoon of FLIPR Potassium Assay principal.

- The assay exploits the permeability of thallium (I) (Tl^+) for potassium (K^+) channels using a novel fluorescent Tl^+ indicator.
- The AM ester form of the dye is loaded into cells in the presence of a special masking dye in the formulation.
- The cells are stimulated with either a mixture of K^+ and Tl^+ (voltage-gated channel, etc.) or a ligand in the presence of Tl^+ (ligand-gated channel).
- The increase of fluorescence recorded using either FLIPR Tetra or FlexStation® 3 Instrument represents the influx of Tl^+ into the cell specifically through the potassium channel.

MATERIALS & METHODS

- The FLIPR Potassium Assay Kit (Molecular Devices) contains Tl^+-sensitive dye, masking dye for homogenous operation, 200mM K_2SO_4, 50 mM Tl_2SO_4, and buffers. Manufacturer protocols are included for both 96- and 384-multiwell plates.
- hERG channel assay: Chinese hamster ovary (CHO) cells stably transfected with human K_V11.1 channel were provided by ChanTest Corporation (Cleveland, OH). Reference hERG blockers used in this study were obtained from Sigma-Aldrich (St. Louis, MO)
- K_V1.3 cell line: Chinese hamster ovary (CHO) cells were stably transfected with human K_V1.3 channel (Molecular Devices, LLC).
- The non-homogeneous potassium ion channel assay was run using FluxOR Assay Kit (Life Technologies, Carlsbad, CA) following the manufacturer's protocol.
- All plates were run on FLIPR Tetra Systems using the Calcium Assay setup (Ex 485nm, Em 530nm). Data were acquired at 1- or 2-second intervals for 140 to 200 seconds.

ASSAY WORKFLOW

Start with overnight culture of confluent monolayer cells in 384-well plates

FLIPR Potassium Assay Kit

1. (Remove cell media, optional)
2. Add 25 µL/well dye solution (if no media removal) Incubate at RT or 37°C for 1 – 1.5 hrs
3. Add 10 µL of agonist or antagonist Incubate at RT or 37°C for 20 min
4. Add 10 µL stimulus & thallium Measure signals on FLIPR Tetra Instrument

Non-Homogeneous K^+ Assay Kit

1. Remove cell media
2. Add 20 µL/well dye solution Incubate at RT or 37°C for 1 – 1.5 hrs
3. Replace with 20 µL Assay Buffer or Wash with 50 µL HEPES buffered HBSS then add 20 µL Assay Buffer *
4. Add 5 µL agonist or antagonist Incubate at RT or 37°C for 20 min
5. Add 5 µL stimulus & thallium Measure signals on FLIPR Tetra Instrument

* Extra wash step used for high-throughput assay validation (Fig. 4 and 5)

ASSAY PERFORMANCE RESULTS

K_V1.3 Channel Activity: Assay optimization with Tl^+/K^+ Titration

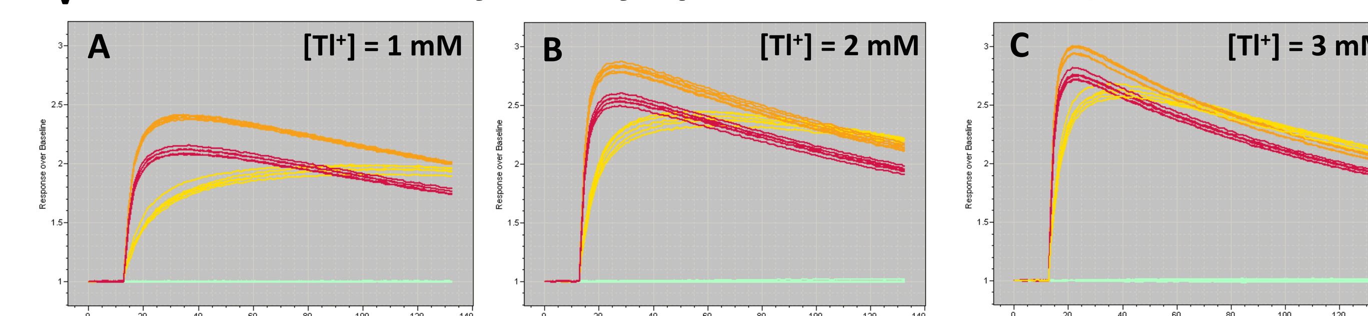
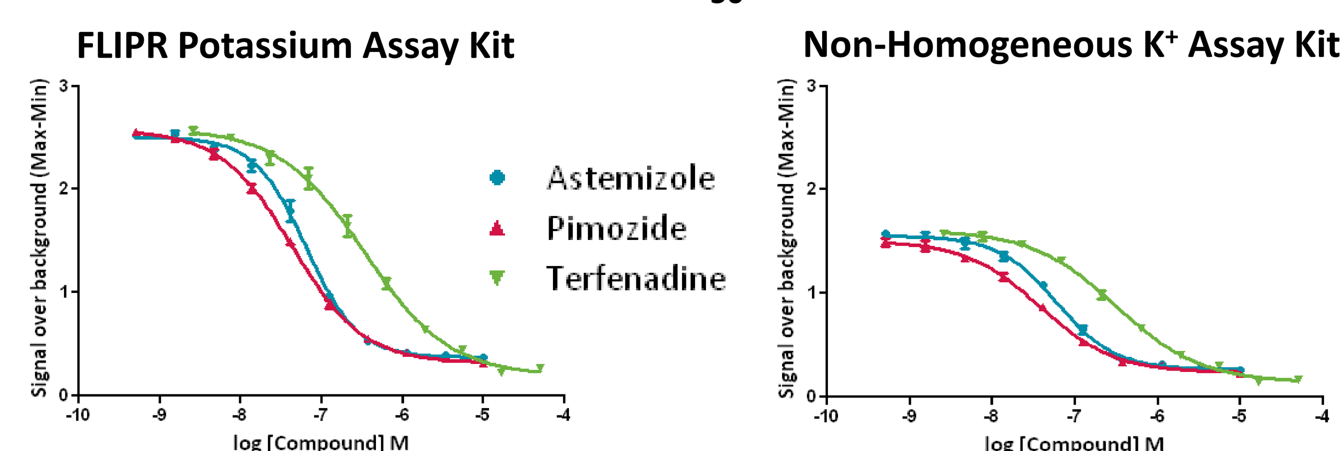


Figure 2. K_V1.3 channel activity was measured with different K^+ and Tl^+ concentrations for optimal assay performance. [K^+] = 10 mM (—), 20 mM (—), or 30 mM (—) was used as stimulus in the presence of 1 mM Tl^+ (A), 2 mM Tl^+ (B) and 3 mM Tl^+ (C). Data were normalized to non-stimulated condition at 0 mM K^+ (—).

hERG Channel Pharmacology – IC_{50} Determination of Blockers

FLIPR Potassium Assay Kit | **Non-Homogeneous K^+ Assay Kit**



IC_{50} nM	FLIPR Potassium Assay	Non-Homogeneous Assay
Astemizole	64	60
Primozide	43	38
Terfenadine	337	316

Figure 3. IC_{50} Determination of hERG channel blockers using the FLIPR Potassium Assay kit versus a non-homogeneous potassium assay kit. Cell media was removed to prevent potential serum interference of the IC_{50} determination. Cells were dye-loaded for 1 hour at RT. The dye solution was replaced with assay buffer for the non-homogeneous assay. Compounds were then incubated with cells for 25 min at RT after dye-loading. The assay was carried out using 1 mM Tl^+ and 10 mM K^+ as stimulus.

HIGH-THROUGHPUT ASSAY VALIDATION RESULTS

Assay Development for HTS of Activators of a Voltage-Gated Potassium Channel – Compound Concentration Titration

FLIPR Potassium Assay Kit | **Non-Homogeneous K^+ Assay Kit**

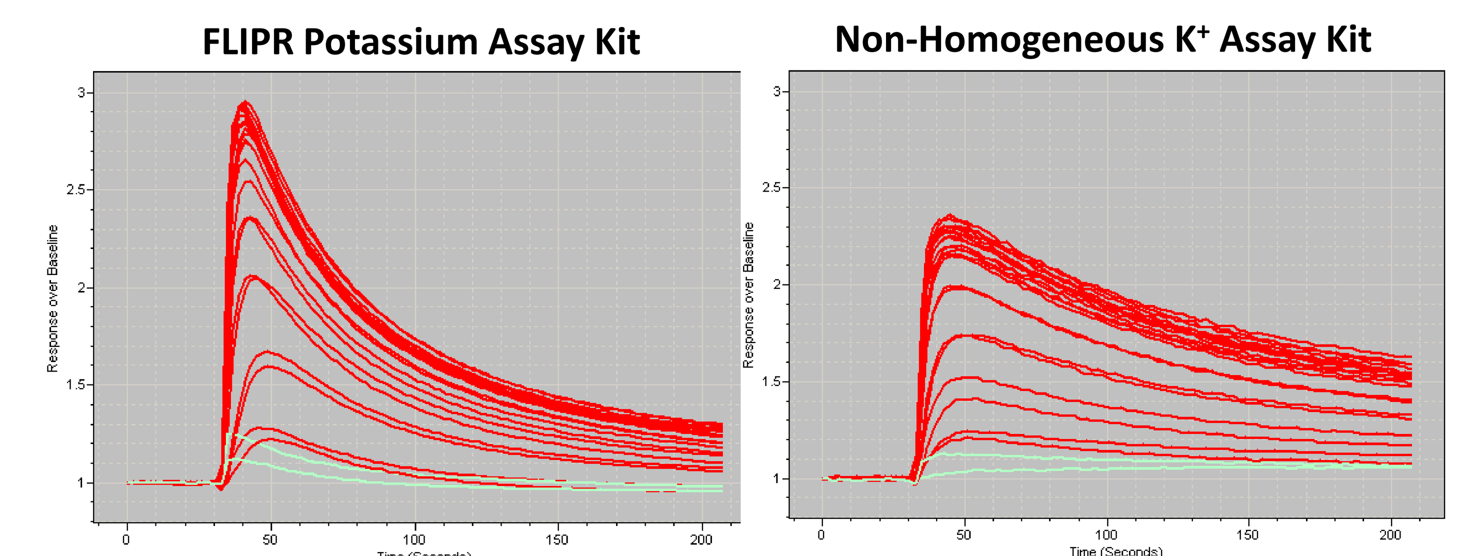
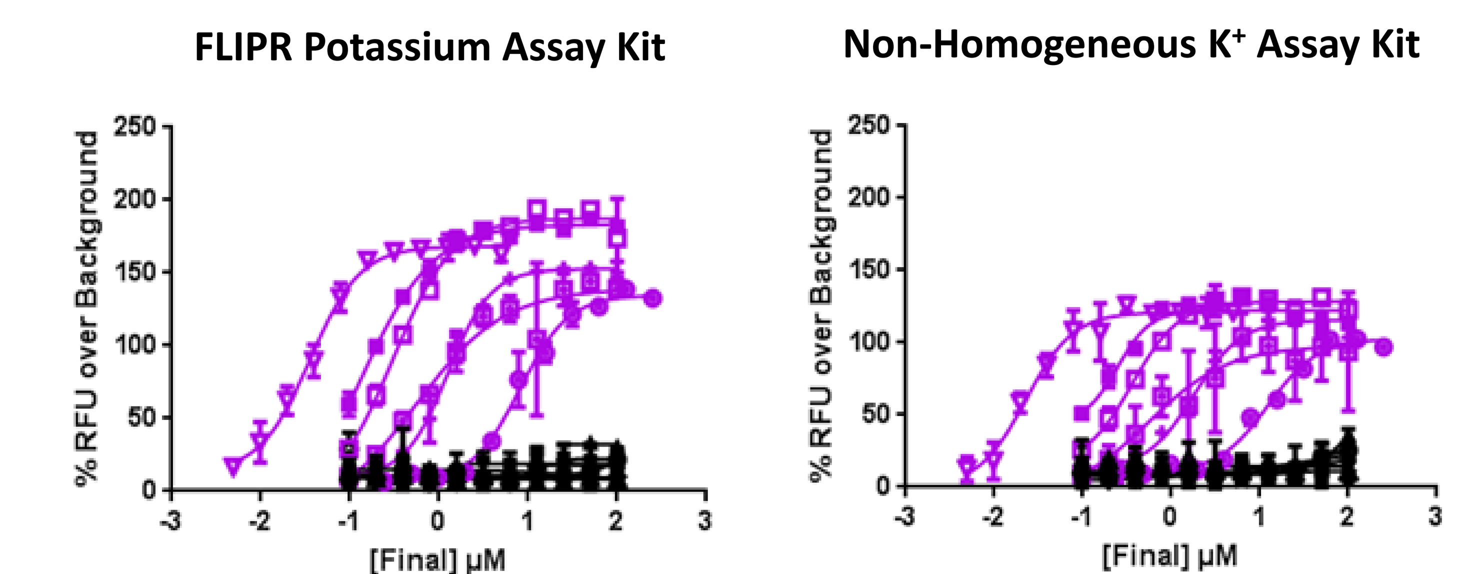


Figure 4. Activation of a voltage-gated potassium channel by Compound X. Signal traces acquired on the FLIPR Tetra Instrument show the ion channel activity induced by a concentration titration of a known Compound X in the presence of 2 mM Tl^+ (n = 2). Cells were dye loaded at 37°C for 90 min. Compound X of a serial dilution was then incubated with the cells for 20 min at 37°C. 2 mM Tl^+ (final concentration) was added to start the assay measurement. Data were normalized to non-stimulated control wells (green).

Assay Validation for HTS of Activators of a Voltage-Gated Potassium Channel – Compound EC_{50} Determination



	FLIPR K^+ Assay Kit		Non-Homogeneous K^+ Assay Kit	
	Max (%)	EC_{50} (µM)	Max (%)	EC_{50} (µM)
A ■	184	0.128	122	0.247
B □	188	0.305	128	0.360
C ▽	168	0.0355	121	0.0226
D ✚	153	1.26	116	1.80
E ●	135	8.07	103	12.3
F ▩	139	0.550	98	0.561

Figure 5. The FLIPR Potassium Assay Kit shows similar IC_{50} values to the non-homogeneous assay but has a significantly higher assay window, as indicated by Max (%) values, and smaller error bars. The assay protocol was the same as that described in Figure 4.

Summary

- The FLIPR Potassium Assay Kit provides functional measurement of K^+ channel activities
- The homogeneous no wash protocol enhances ease-of-use and reduces total assay time
- The kit shows reduced well-to-well variation and improved data quality compared to non-homogeneous formats

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