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

The increase of fluorescence recorded using FLIPR Tetra or FlexStation® 3 when cells are stimulated with either a mixture of K+ and Tl+ (voltage-gated channel, •) or a ligand in the presence of Tl+ (ligand-gated channel). The assay exploits the permeability of thallium (I) (Tl+) for potassium (K+) channels. 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 WORKFLOW

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

FLIPR Potassium Assay Kit

(Remove cell media, optional)

Add 25 µL/well dye solution (if no media removal)

Incubate at RT or 37°C for 1–1.5 hrs

Add 10 µL of agonist or antagonist

Incubate at RT or 37°C for 20 min

Add 5 µL of agonist or antagonist

Incubate at RT or 37°C for 20 min

Measures signals on FLIPR Tetra Instrument

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

ASSAY PERFORMANCE RESULTS

Kv1.3 Channel Activity: Assay optimization with Tl+/K+ Titration

A

[|Tl+| = 1 mM] B C

Add 10 µL of agonist or antagonist

Incubate at RT or 37°C for 20 min

Add 5 µL of agonist or antagonist

Incubate at RT or 37°C for 20 min

Measures signals on FLIPR Tetra Instrument

Figure 2. Kv1.3 channel activity was measured with different K+ and Tl+ concentrations for optimal assay performance. [K+] = 10 mM (…), 20 mM (…), or 30 mM (…). Data were normalized to non-stimulated control wells (green).

hERG Channel Pharmacology – IC50 Determination of Blockers

FLIPR Potassium Assay Kit

Non-Homogeneous K+ Assay Kit

Figure 3. IC50 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 IC50 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.

ASSAY DEVELOPMENT RESULTS

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

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 RESULTS

Figure 5. The FLIPR Potassium Assay Kit shows similar IC50 values to the non-homogeneous assay but has a significantly higher assay window, as indicated by Max (%)

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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