Thallium-Free hERG Potassium Channel Assay

Catalog No. 12100

Introduction

Human Ether-à-go-go-Related Gene (hERG) potassium channels, also called K_V11.1 and encoded by the KCNH2 gene, are voltage-gated channels essential for repolarizing the cardiac action potential. These channels are made up of four α-subunits that create a selective pore for potassium ions and open when the membrane potential changes. Dysfunction or pharmacological inhibition of hERG channels leads to delayed cardiac repolarization, manifesting as drug-induced long QT syndrome which is a potentially fatal arrhythmia of major concern in drug development. Because of this, regulatory agencies routinely screen new drug candidates for K_V11.1 inhibition.

Traditional methods to assess hERG channel activity such as patch clamp electrophysiology, FRET-based assays, or thallium flux can be labor-intensive, low-throughput, or dependent on toxic surrogate ions. To facilitate more efficient and physiologically relevant evaluation of hERG activity, ION Biosciences has developed a Thallium-Free hERG Potassium Channel Assay utilizing ION Potassium Green 1 AM (IPG-1 AM), a membrane-permeable, fluorescent dye sensitive to intracellular potassium levels. Upon membrane depolarization, hERG channels open and mediate potassium efflux, causing a decrease in intracellular potassium concentration. This drop is detected in real time as a reduction in IPG-1 fluorescence intensity, providing a direct measure of channel function.

Designed for high-throughput screening, the assay is compatible with 96- and 384-well plate formats and enables rapid assessment of hERG modulators. The Thallium-Free hERG Potassium Channel Assay provides reliable EC_{50}/IC_{50} values and is ideally suited for pharmacological profiling, cardiotoxicity safety screening, and therapeutic discovery targeting hERG channels.

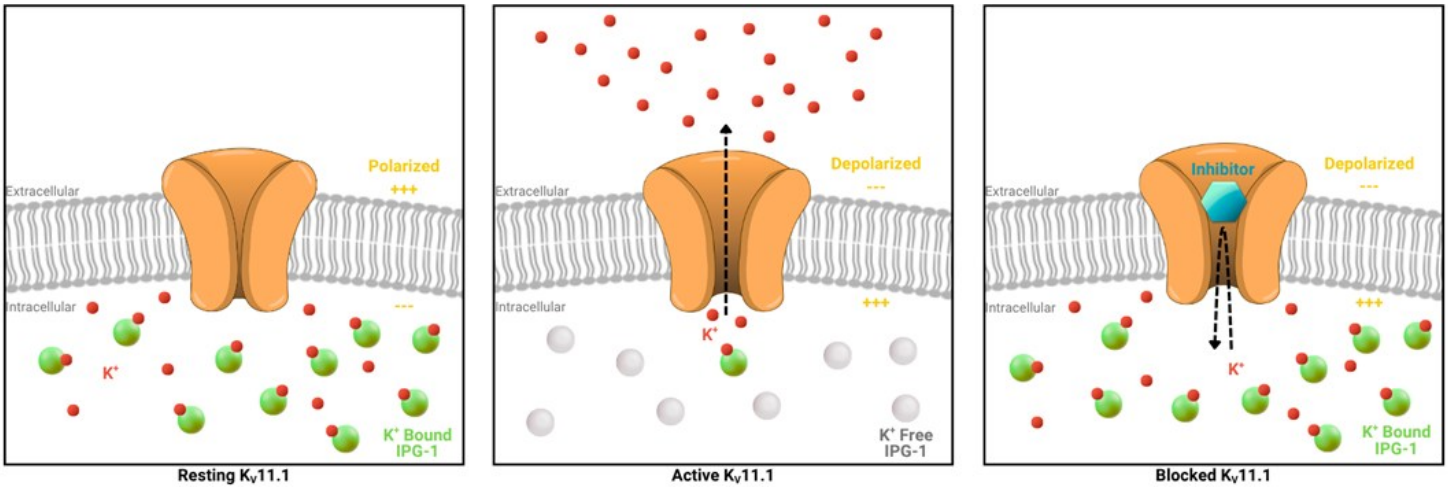

Figure 1. Thallium-Free HERG Potassium Channel Assay Principle. Cells expressing hERG channels are incubated with a Dye Loading Solution containing IPG-1 AM, a fluorescent indicator sensitive to potassium. Activation of hERG channels through membrane depolarization induces rapid potassium efflux, resulting in a decrease in intracellular potassium. This change is detected as a reduction in fluorescence, enabling a direct measurement of hERG channel activity. When the channel is pre-treated with an inhibitor, depolarization-induced potassium efflux is attenuated.

Storage and Stability

Upon receipt, store components at the temperatures indicated on each label. Kit components are stable[1] for up to 12 months from date of shipment when stored as directed.

Table 1	Kit Contents	*Flex* Kit Cat# 12100-10		*pIONeer* Kit Cat# 12100-2		
Label	**Name**	**Size**	**Qty**	**Size**	**Qty**	**Storage**
Reagent A	IPG-1 AM	50 µg Vial	10	50 µg Vial	2	-20° C
Reagent B	DMSO[1]	225 µL Vial	1	Not included in pIONeer		-20° C
Reagent C	50X DySolv	4 mL Bottle	1	800 µL Vial	1	4° C
Reagent D	10X hERG Potassium Assay Buffer	30 mL Bottle	1	6 mL Bottle	1	4° C
Reagent E	50X TRS[2]	4 mL Bottle	1	800 µL Vial	1	4° C
Reagent F	50X Probenecid[2]	4 mL Bottle	1	800 µL Vial	1	4° C
Reagent G	10X hERG Potassium Stimulus Buffer	10 mL Bottle	1	2 mL Bottle	1	4° C

Getting Started

Before beginning your experiment, ensure you have all the necessary reagents and materials. ION Biosciences' Thallium-Free hERG Potassium Channel Assay includes everything required for the assay, except for hERG Channel modulators and hERG-expressing cell lines (also available from ION Biosciences, contact sales@ionbiosciences.com for more information). A fluorescence plate reader with excitation capabilities between 485–525 nm and emission collection around 545 nm is also needed; ideally, the reader should support kinetic data collection at about 1 Hz. Examples of suitable instruments include the WaveFront Panoptic, Hamamatsu FDSS, Molecular Devices FLIPR, and Molecular Devices FlexStation.

These instructions are designed for both 96-well and 384-well microplate formats. Adjustments may be needed for other formats, such as 1536-well plates or non-adherent cells. IPG-1 AM should be protected from direct light and solutions containing IPG-1 AM should be used within 2 hours of preparation. We recommend seeding hERG-expressing cells in 96-well or 384-well plates 24 hours prior to the assay, with 20K cells per well for 96-well plates and 10K cells per well for 384 -well plates.

Laboratory Procedures

1. Add 20 µL of DMSO (**Reagent B**) to the tube containing IPG-1 AM (**Reagent A**).
2. Vortex until Reagent A is fully dissolved.
3. Add appropriate volume of water (**Table 2**, next page) to a 15 mL centrifuge tube.
4. Add 1 mL of 10X hERG Potassium Assay Buffer (**Reagent D**) to the tube from **step 3**.

Procedure Continues on Next Page

Laboratory Procedures (Continued)

5. Add 200 µL of 50X DySolv (**Reagent C**) to the tube from **step 4**.
6. Add 200 µL of 50X TRS[2] (**Reagent E**) to the tube from **step 5**.
7. If desired add 200 µL of 50X Probenecid[2] (**Reagent F**) to the tube from **step 6**.
8. Add 20 µL of IPG-1 AM Solution from **step 2** to the tube from **step 7**.
9. Briefly vortex the **Dye Loading Solution** from **step 8** to mix well.

Table 2	**Dye Loading Solution**		
Label	**Name**	**Method A**	**Method B**
Reagent A+B	IPG-1 AM solution in DMSO	20 µL	20 µL
Reagent C	50X DySolv	200 µL	200 µL
Reagent D	10X hERG Potassium Assay Buffer	1 mL	1 mL
Reagent E	50X TRS[2]	200 µL	200 µL
Reagent F	50X Probenecid[2]	200 µL	-
	Water	8.4 mL	8.6 mL
	Total	**10 mL**	**10 mL**

10. Completely remove the cell-culture medium from the 96-well or 384-well microplate containing the cells of interest.
11. Prepare the **Wash Solution**[3] in a 15 mL centrifuge tube by adding the appropriate amounts of water and 10X hERG Potassium Assay Buffer (**Reagent D**) as shown in **Table 3** below. Briefly vortex to mix well.
12. Wash the cell-containing microplate once with **Wash Solution**[3]. 100 µL/well for 96-well plate and 20 µL/well for 384-well plate. Completely remove the **Wash Solution**[3] from the 96-well or 384-well plates.

Table 3	**Wash Solution³ (Optional)**	
Label	**Name**	**Volume**
Reagent D	10X hERG Potassium Assay Buffer	1 mL
	Water	9 mL
	Total	**10 mL**

13. Add the **Dye Loading Solution** from **step 9** to the cell-containing microplate. 100 µL/well for 96-well plate and 20 µL/well for a 384-well plate.
14. Incubate the cell-containing microplate with the **Dye Loading Solution** for 60 minutes at 37°C.

Procedure Continues on Next Page

Laboratory Procedures (Continued)

15. Prepare the Inhibitor Solution in a 15 mL centrifuge tube by adding the appropriate amounts of water, 10X hERG Potassium Assay Buffer (**Reagent D**), and 3X concentrated hERG Channel inhibitors as shown in **Table 4** below. Briefly vortex to mix well.
16. Add the **Inhibitor Solution** from **step 15** to the cell-containing microplate. 50 µL/well for a 96-well plate and 10 µL/well for a 384-well plate.
17. Incubate the cell-containing microplate for 15 minutes at 37°C.

Table 4	**Inhibitor Solution**	
Label	**Name**	**Volume**
Reagent D	10X hERG Potassium Assay Buffer	1 mL
(Customer Supplied Material)	hERG Channel Inhibitors	Enough for 3X concentration at total volume
	Water	Q.S. to total volume
	Total	**10 mL**

18. Prepare the **Stimulus Solution**[4] in a 15 mL centrifuge tube by adding the appropriate amounts of water, 10X hERG Potassium Assay Buffer (**Reagent D**), and 10X hERG Potassium Stimulus Buffer (**Reagent G**) as shown in **Table 5** below. Briefly vortex the **Stimulus Solution**[4] to mix well. The concentration of 10X hERG Potassium Stimulus Buffer in the **Stimulus Solution**[4] should be optimized for each assay.
19. Load the **Stimulus Solution**[4] from **step 18** into a V-shaped reagent plate for assay readout. 100 µL/well for a 96-well

Table 5	**Stimulus Solution** [4]			
Label	**Name**	**Example A**	**Example B**	**Example C**
Reagent D	10X hERG Potassium Assay Buffer	-	0.5 mL	0.75 mL
Reagent G	10X hERG Potassium Stimulus Buffer	1 mL	0.5 mL	0.25 mL
	Water	9 mL	9 mL	9 mL
	Total	**10 mL**	**10 mL**	**10 mL**

20. Transfer the cell-containing microplate and reagent plate to a kinetic-imaging plate reader (e.g. WaveFront Panoptic, Hamamatsu FDSS, Molecular Devices FLIPR, or Molecular Devices FlexStation).
21. Acquire data using an excitation wavelength of ~515 nm, an emission wavelength of ~545 nm, and an acquisition frequency of ~1 Hz. Excitation sources commonly used for fluorescein, Fluo-4, and GFP (480-490 nm) are compatible with ION's IPG-1 AM fluorescent dye. See **Table 6** (next page) for recommended instrument settings.

Procedure Continues on Next Page

Laboratory Procedures (Continued)

21. Begin baseline data acquisition for 1 minute, then transfer the volume from the reagent plate to the cell-containing microplate. 50 µL/well for a 96-well plate and 10 µL/well for a 384-well plate. Continue data acquisition for an additional 30 - 60 minutes.

Table 6	Recommended Instrument Settings
Setting	**Recommendation**
Read Mode	'Bottom' read mode only
Ex/Em wavelengths[5]	~515 nm/545 nm
Cutoff wavelength	530 nm
Filter selection	GFP, FITC, YFP
Contact support@ionbiosciences.com for additional recommendations and guidance on optimizing to your application.	

Laboratory Procedures - Footnotes

1 DMSO is hygroscopic and should be stored tightly closed at -20° C with desiccant pack to prevent the solvent from becoming wet. Wet solvent causes difficulties with dissolution of the dye. Use the DMSO within 6 months of receipt.

2 Caution is advised when using Probenecid and/or TRS as they may have undesirable effects on assay performance for the target of interest. Probenecid may be included in the Dye Loading Solution to aid in dye retention. This may be particularly important in certain cell lines (e.g. CHO-K1 cells). TRS contains a membrane-impermeant dye useful for masking extracellular fluorescence.

3 We recommend washing the cells with wash solution only if residual culture medium remains in the well.

4 The timing and volume of stimulus solution addition may vary. Some experiments may include the addition of other solutions to the cell-containing microplate prior to the addition of the stimulus solution. In these cases, the volume of the stimulus solution addition should be altered to account for the additional volume of solution in the cell-containing microplate.

5 To prevent bleed-through or spectral overlap, the Ex/Em wavelengths may need to be optimized by broadening the interval between the wavelengths. Results displayed were generated on a Wavefront Panoptic.

Data Analysis

1. Plot the relative fluorescence units (F) over time as the raw kinetic fluorescence data (**Figure 2**).
2. Define the initial 60 seconds of relative fluorescence units (F) as the baseline. Average the baseline and define it as F_0. Calculate the ratio of F/F_0 at each time point. Plot F/F_0 over time as the baseline-normalized kinetic fluorescence data (**Figure 3**).
3. Use GraphPad® Prism (or another similarly capable graphing and statistical analysis software) to apply an Area Under Curve (AUC) analysis on the baseline-normalized kinetic fluorescence data by measuring the area between the curve and the baseline at y=1. Plot the AUC against hERG Channel inhibitor concentration to make a concentration response curve (**Figure 4**).
4. Using GraphPad® Prism, run the non-linear regression "inhibitor vs response" to calculate the half-maximal inhibitory concentration (IC_{50}) (**Figure 5**). Alternatively, run the non-linear regression "agonist vs response" to calculate the half-maximal effective concentration (EC_{50}).

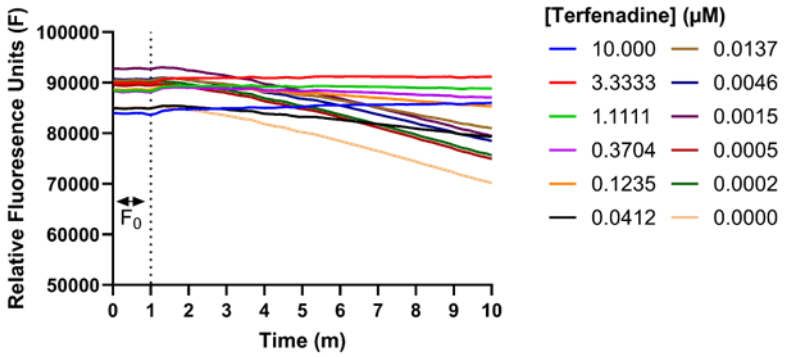

Figure 2. Raw Kinetic Fluorescence Data. Raw fluorescence data of $K_V11.1$-expressing HEK293 cells collected using Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm). Terfenadine, a selective hERG channel inhibitor, was added 15 minutes before the start of the assay. Relative Fluorescence Units (F) were collected for 60 seconds before the addition of the Stimulus Solution. After addition, the plate continued to be read for an additional 60 minutes. The first 10 minutes of data are shown.

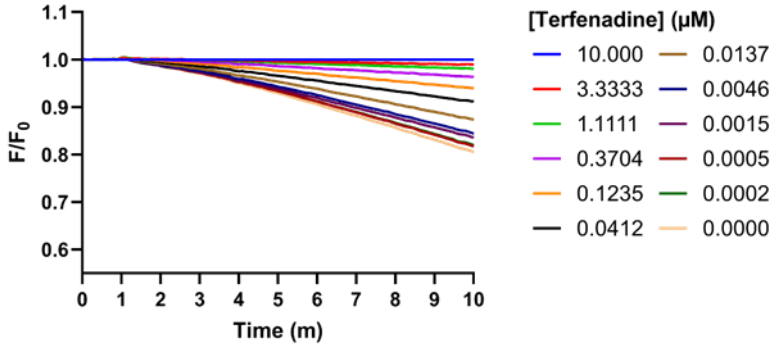

Figure 3. Baseline-Normalized Fluorescence Kinetic Data. Based on the Raw Kinetic Fluorescence Data from **Figure 2**, average the Relative fluorescence Units (F) of the initial 60 seconds before stimulus addition as the baseline (F_0). Calculate the ratio of Relative fluorescence Units (F) over F_0 at each time point as F/F_0. Plot F/F_0 over time as the Baseline-Normalized Fluorescence Kinetic Data.

Data Analysis (Continued)

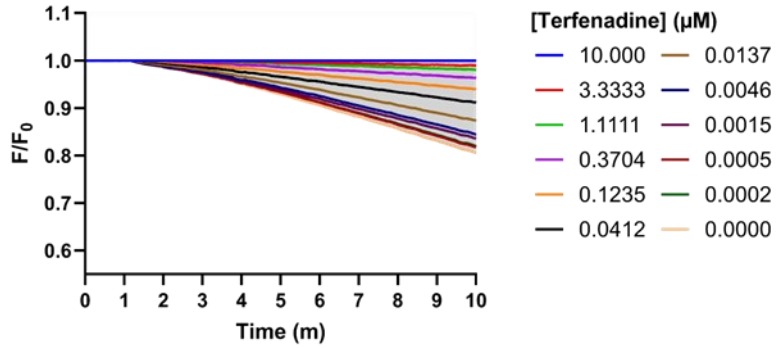

Figure 4. Area Under Curve (AUC) Calculation. Based on the Baseline-Normalized Fluorescence Kinetic Data from **Figure 3**, calculate the area between the curve and y=1 and define it as the AUC.

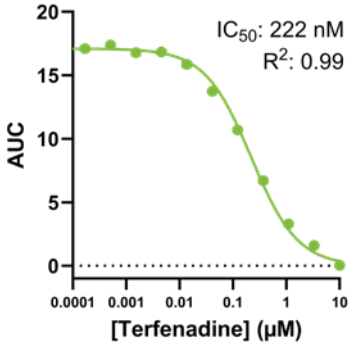

Figure 5. IC_{50} Calculation. Plot the AUC against the compound concentrations and run the non-linear regression "inhibitor vs response" in GraphPad® Prism to calculate an IC_{50}. Alternatively, run the non-linear regression "agonist vs response" to calculate an EC_{50}.

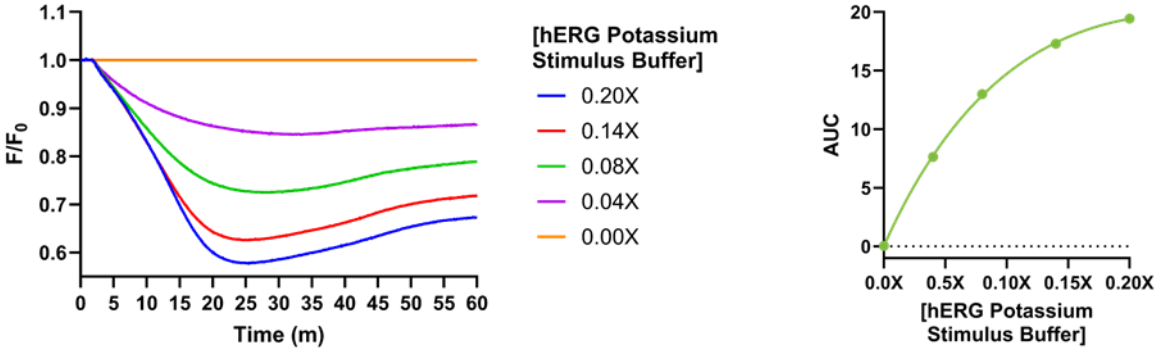

Figure 6. Activation of hERG Channels in K_V11.1-Expressing HEK293 Cells. Baseline-normalized fluorescence data collected using Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm). Stimulus Solutions containing various concentrations of hERG Potassium Stimulus Buffer were added at 60 seconds. Reported values are the final concentration of stimulus solution in the well relative to the provided 10X buffer stock. The AUC was calculated between the curve and y=1. Error bars indicate the SEM (n = 3). Increasing concentrations of hERG Potassium Stimulus Buffer will increase K_V11.1 activation.

Example Results

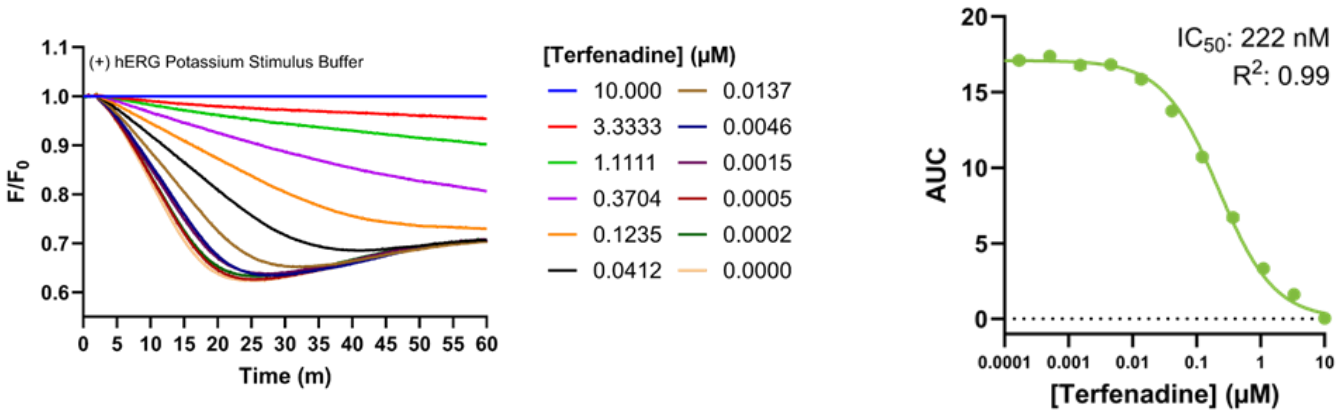

Figure 7. Terfenadine Inhibition of hERG Channels in K_V11.1-Expressing HEK293 Cells. Baseline-normalized fluorescence data collected using Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm). Terfenadine, a potent hERG channel inhibitor, was added 15 minutes before the start of the assay. hERG Potassium Stimulus Buffer was added at 60 seconds at a concentration of 0.14X. The AUC was calculated between the curve and y=1. Error bars indicate the SEM (n = 3). The IC_{50} for Terfenadine inhibition of K_V11.1 is about 222 nM.

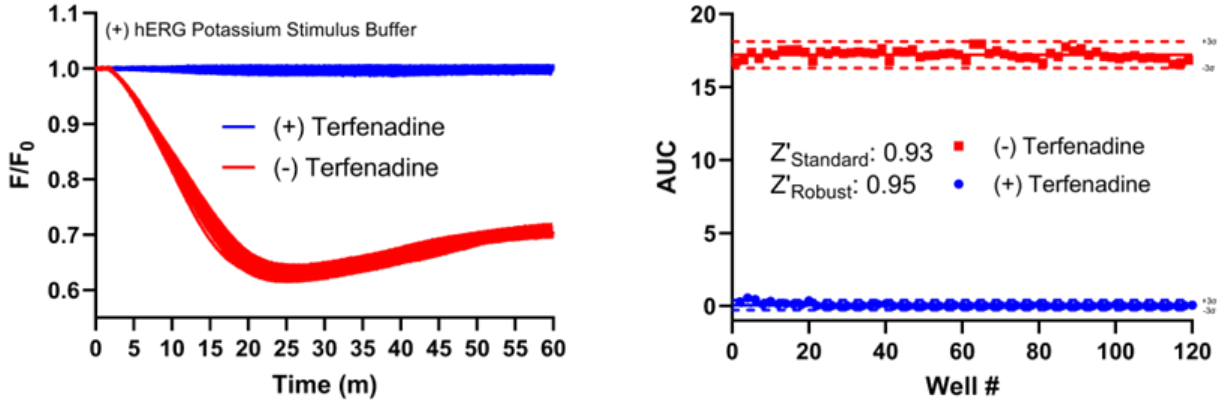

Figure 8. Z' Determination in K_V11.1-Expressing HEK293 Cells. Baseline-normalized fluorescence data collected using Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm). ±Terfenadine, a potent hERG Channel inhibitor, was pre-incubated for 15 minutes at a concentration of 10 μM before the start of the assay. hERG Potassium Stimulus Buffer was added at 60 seconds at a concentration of 0.14X. The AUC was calculated between the curve and y=1. The Z' factor plots show a Z' above 0.9 for terfenadine inhibition of K_V11.1 (n = 60).

Example Results (Continued)

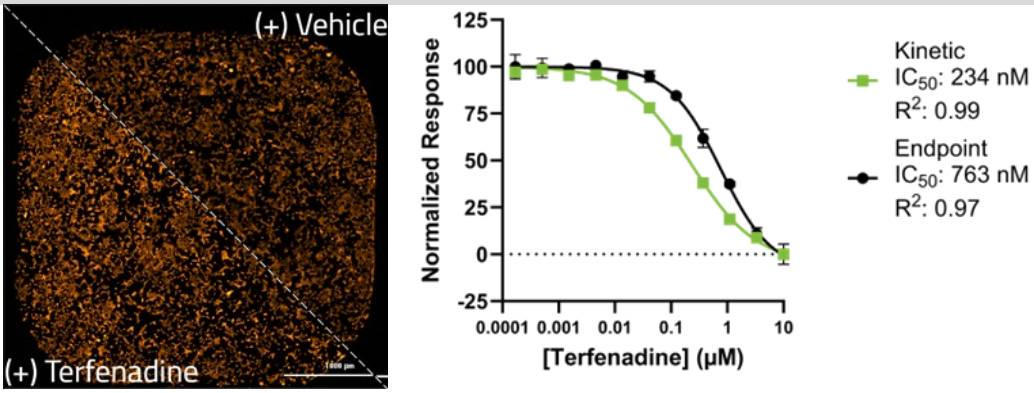

Figure 9. Endpoint Readout of K_V11.1-Expressing HEK293 Cells. Whole-well images were acquired using Cytation 5 imager with a 4X objective and RFP filter (Excitation: 531(40) nm, Emission: 593(40) nm). Bottom-read data were collected using Cytation 5 monochromators (Excitation: 515(9) nm, Emission: 545(9) nm). Terfenadine, a potent hERG channel inhibitor, was added 15 minutes before the start of the assay. hERG Potassium Stimulus Buffer was added at a concentration of 0.14X and incubated for 1 hour at room temperature before reading. Error bars indicate the SEM (n = 3). The IC_{50} for Terfenadine inhibition of K_V11.1 is similar between kinetic and endpoint mode.

Additional Information

Additional dye indicator and buffer reagents can be purchased either directly from our website or by contacting our Sales Department. Custom and bulk sizes are also available. Contact Sales for more information.

Table 7	Additional Reagents	Available Sizes	
Kit Label	**Name**	**Size**	**Catalog #**
Reagent A	IPG-1 AM	500 µg x 1 Vial 50 µg x 10 Vials 50 µg x 3 Vials	3041C 3041F 3041G
Reagent C	50X DySolv	20 mL Bottle	7501A
Reagent D	10X hERG Potassium Assay Buffer	Not a Regular Catalog Item. Contact Sales About Additional Purchase.	
Reagent E	50X TRS	20 mL Bottle	7060A
Reagent F	50X Probenecid	20 mL Bottle	7300P-50
Reagent G	10X hERG Potassium Stimulus Buffer	Not a Regular Catalog Item. Contact Sales About Additional Purchase.	
hERG Expressing Cell Lines	HEK293 K_V11.1 (hERG) Cell Line CHO-K1 K_V11.1 (hERG) Cell Line	2 x 10^6 Cells x 1 vial 2 x 10^6 Cells x 1 vial	C1103 C1202

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