Thallium-Free GIRK Potassium Channel Assay

Catalog No. 12000

Introduction

G protein-gated inwardly rectifying potassium (GIRK) channels are key regulators of cellular excitability in both the nervous system and the heart. Composed of $K_{ir}3.1$–$K_{ir}3.4$ subunits, GIRK channels are activated by $G_{\beta\gamma}$ subunits released from $G_{i/o}$-coupled G protein-coupled receptors (GPCRs), including dopamine, serotonin, muscarinic, and opioid receptors. In the central nervous system, the predominant functional form is the $K_{ir}3.1/K_{ir}3.2$ heterotetramer, which plays a critical role in modulating neuronal firing. GIRK dysfunction has been linked to a wide range of neurological and cardiac disorders, including epilepsy, Parkinson's disease, drug addiction, and atrial fibrillation, making it a compelling target for drug discovery.

Traditional approaches for measuring GIRK activity such as patch clamp, FRET, or thallium flux assays can be labor-intensive, low-throughput, or reliant on toxic surrogate ions. To overcome these limitations, ION Biosciences has developed the Thallium-Free GIRK Potassium Channel Assay featuring ION Potassium Green 1 AM (IPG-1 AM), a membrane-permeable, potassium-sensitive, fluorescent indicator that directly measures potassium flux.

Upon binding of an agonist to $G_{i/o}$-coupled GPCRs or direct activation of GIRK channels, potassium ions flow through the channel and out of the cell, resulting in a measurable decrease in intracellular potassium concentration. This change is detected in real-time by IPG-1 as a loss of fluorescence intensity. This direct-readout approach eliminates the need for surrogate ions or radioactive tracers and enables robust, high-throughput screening of GIRK modulators in 96- and 384-well formats. The Thallium-Free GIRK Potassium Channel Assay provides reliable EC_{50}/IC_{50} values and is ideally suited for pharmacological profiling and discovery of therapeutic compounds targeting GIRK channels or GIRK channel-coupled pathways.

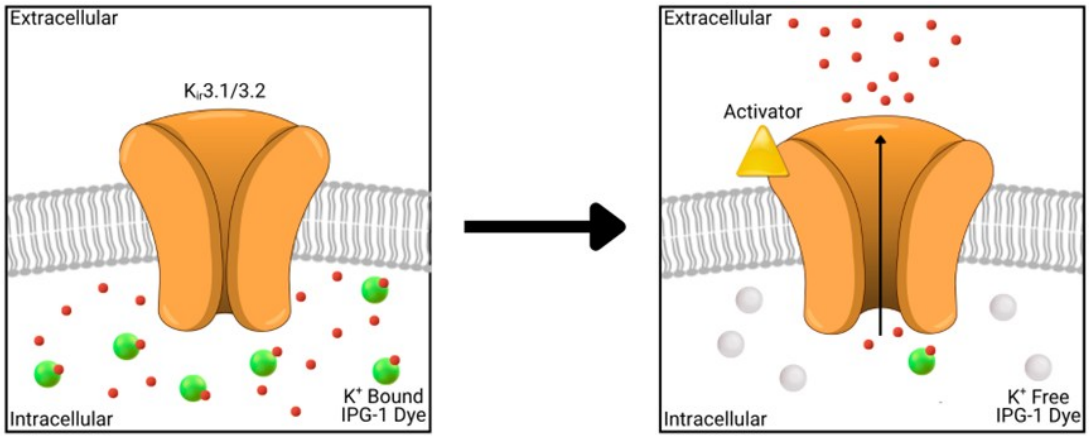

Figure 1. Thallium-Free GIRK Potassium Channel Assay Principle. Cells expressing GIRK channels are incubated with a Dye Loading Solution containing IPG-1 AM, a fluorescent indicator sensitive to potassium. Activation of GIRK channels through $G_{i/o}$-coupled GPCR agonists or direct stimulation induces rapid potassium efflux, resulting in a decrease in intracellular potassium. This change is detected by IPG-1 as a reduction in fluorescence, enabling a direct measurement of GIRK channel activity.

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# 12000-10		*pIONeer* Kit Cat# 12000-2		
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 GIRK Potassium Assay Buffer	40 mL Bottle	1	8 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

Getting Started

Before beginning your experiment, ensure you have all the necessary reagents and materials. ION Biosciences' Thallium-Free GIRK Potassium Channel Assay kit includes everything required for the assay, except for GIRK Channel modulators, substrates, and GIRK-expressing cell lines. 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 GIRK-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 GIRK Potassium Assay Buffer (Reagent D) to the tube from step 3.
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.

Procedure Continues on Next Page

Laboratory Procedures (Continued)

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 GIRK Potassium Assay Buffer	1 mL	1 mL
Reagent E	50X TRS[2]	200 µL	200 µL
Reagent F	50X Probenecid[2]	200 µL	-
	Water	8.6 mL	8.8 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 GIRK 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 Solution[3] (Optional)	
Label	**Name**	**Volume**
Reagent D	10X GIRK 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)

Note: If screening for GIRK channel inhibitors, continue with steps 15–17; otherwise, proceed to Step 18.

15. Prepare the Inhibitor Solution in a 15 mL centrifuge tube by adding the appropriate amounts of water, 10X GIRK Potassium Assay Buffer (Reagent D), and 3X concentrated GIRK 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 (Optional)	
Label	**Name**	**Volume**
Reagent D	10X GIRK Potassium Assay Buffer	1 mL
(Customer Supplied Material)	GIRK 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 GIRK Potassium Assay Buffer (Reagent G), and 4X concentrated GIRK Channel Activators as shown in Table 5 below. Briefly vortex the Stimulus Solution[4] to mix well.
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 plate and 20 µL/well for a 384-well plate.

Table 5	Stimulus Solution [4]	
Label	**Name**	**Volume**
Reagent G	10X GIRK Potassium Assay Buffer	1 mL
(Customer Supplied Material)	GIRK Channel Activators	Enough for 4X concentration at total volume
	Water	Q.S. to total volume
	Total	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).

Procedure Continues on Next Page

Laboratory Procedures (Continued)

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 below for recommended instrument settings.
22. 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 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 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 substrate or modulator concentration to make a concentration response curve (Figure 4).
4. Using GraphPad® Prism, run the non-linear regression "agonist vs response" on the concentration-response curve (V_{max} or AUC) to calculate the half-maximal effective concentration (EC_{50}). Alternatively, run the non-linear regression "inhibitor vs response" to calculate the half-maximal inhibitory concentration (IC_{50}) (Figure 5).

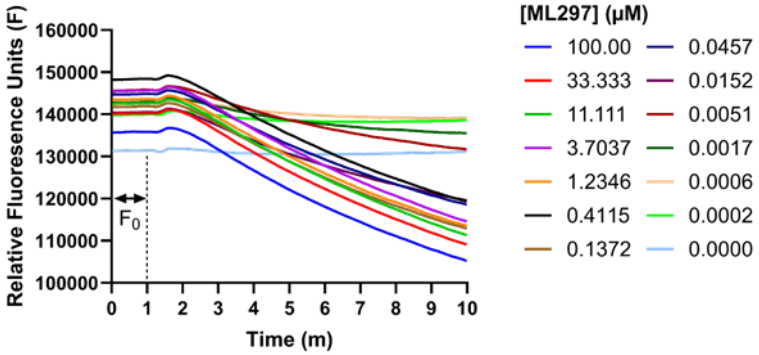

Figure 2. Raw Kinetic Fluorescence Data. Raw kinetic fluorescence data of $K_{ir}3.1/3.2$-expressing HEK293 cells collected using Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm). Relative Fluorescence Units (F) were collected for 1 minute before the stimulus addition of various concentrations of ML297. After addition, the plate continued to be read for an additional 60 minutes. The first 10 minutes of data are shown.

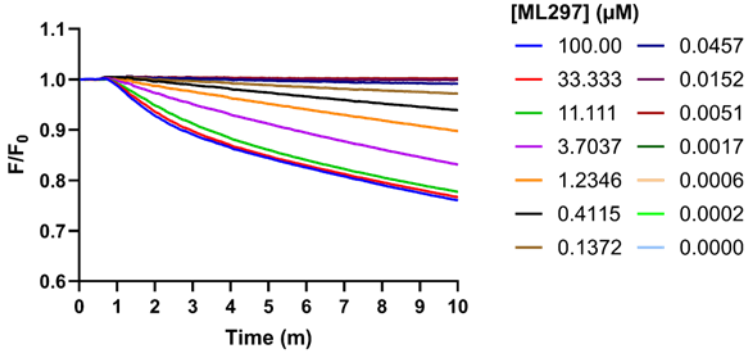

Figure 3. Baseline-Normalized Fluorescence Kinetic Data. Based on the Raw Kinetic Fluorescence Data from figure 2, average the Relative fluorescence Units (F) of the initial 60 seconds before stimulus addition as the baseline (F_0). Calculate the ratio of Relative fluorescence Units (F) over F_0 at each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

Data Analysis (Continued)

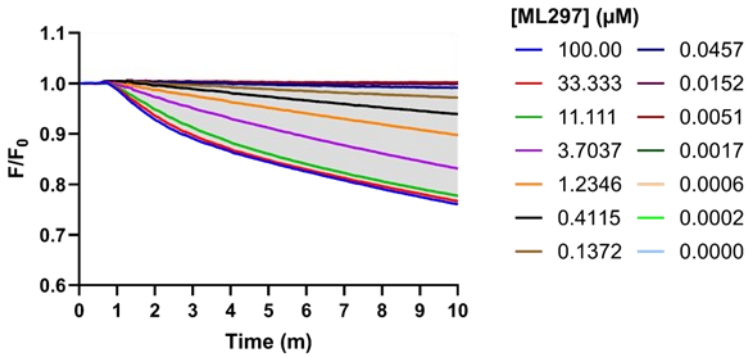

Figure 4. Area Under Curve (AUC) Calculation. Based on the Baseline-Normalized Fluorescence Kinetic Data from Figure 3, calculate the area between the curve and y=1 and define it as the AUC.

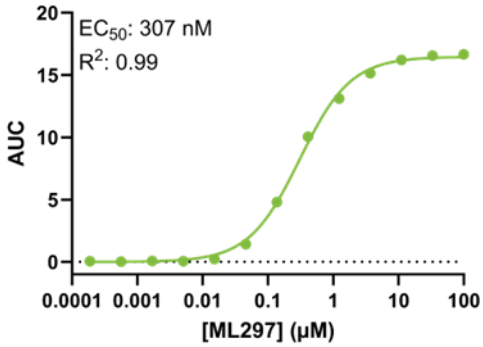

Figure 5. EC_{50} Calculation. Plot the V_{max} or the AUC against the compound concentrations and run the non-linear regression "agonist vs response" in GraphPad® Prism to calculate an EC_{50}. Alternatively, run the non-linear regression "inhibitor vs response" to calculate an IC_{50}.

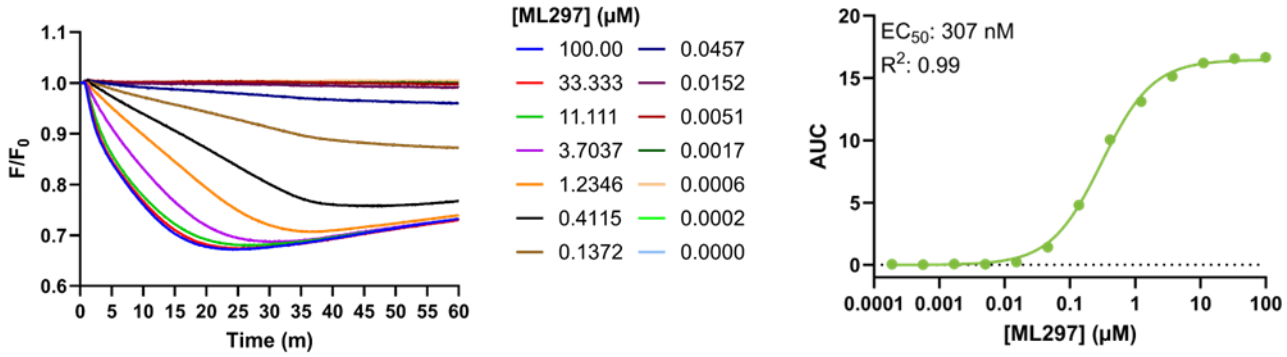

Figure 6. ML297 Activation of GIRK Channels in $K_{ir}3.1/3.2$-Expressing HEK293 Cells. Baseline-normalized kinetic fluorescence data collected using Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm). ML297, a potent activator of GIRK Channels, was added at 60 seconds. The AUC was calculated between the curve and y=1. Error bars indicate the SEM (n = 3). The EC_{50} for ML297 activation of $K_{ir}3.1/3.2$ is about 307 nM.

Example Results

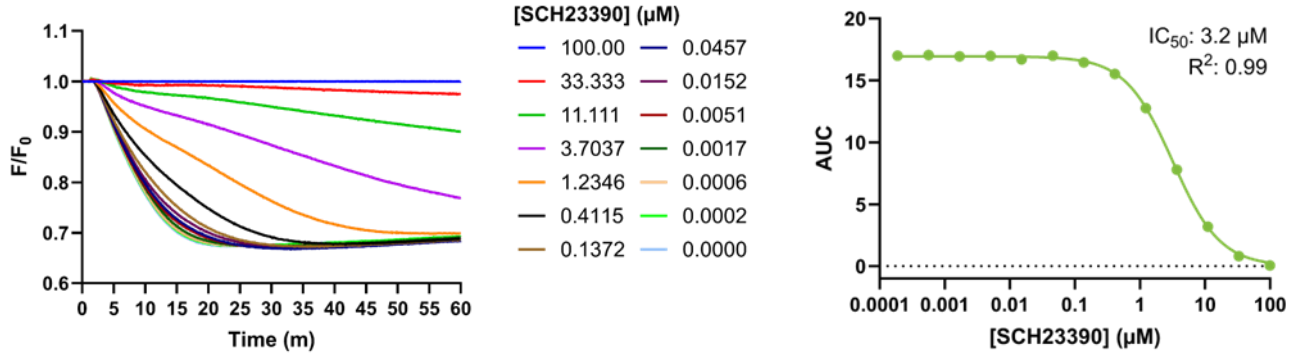

Figure 7. SCH23390 Inhibition of GIRK Channels in K_{ir}3.1/3.2-Expressing HEK293 Cells. Baseline-normalized kinetic fluorescence data collected using Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm). SCH23390, a selective GIRK channel inhibitor, was added 15 minutes before the start of the assay. ML297, a potent activator of GIRK Channels, was added at 60 seconds at a concentration of 10 µM. The AUC was calculated between the curve and y=1. Error bars indicate the SEM (n = 3). The IC_{50} for SCH23390 inhibition of K_{ir}3.1/3.2 is about 3.2 µM.

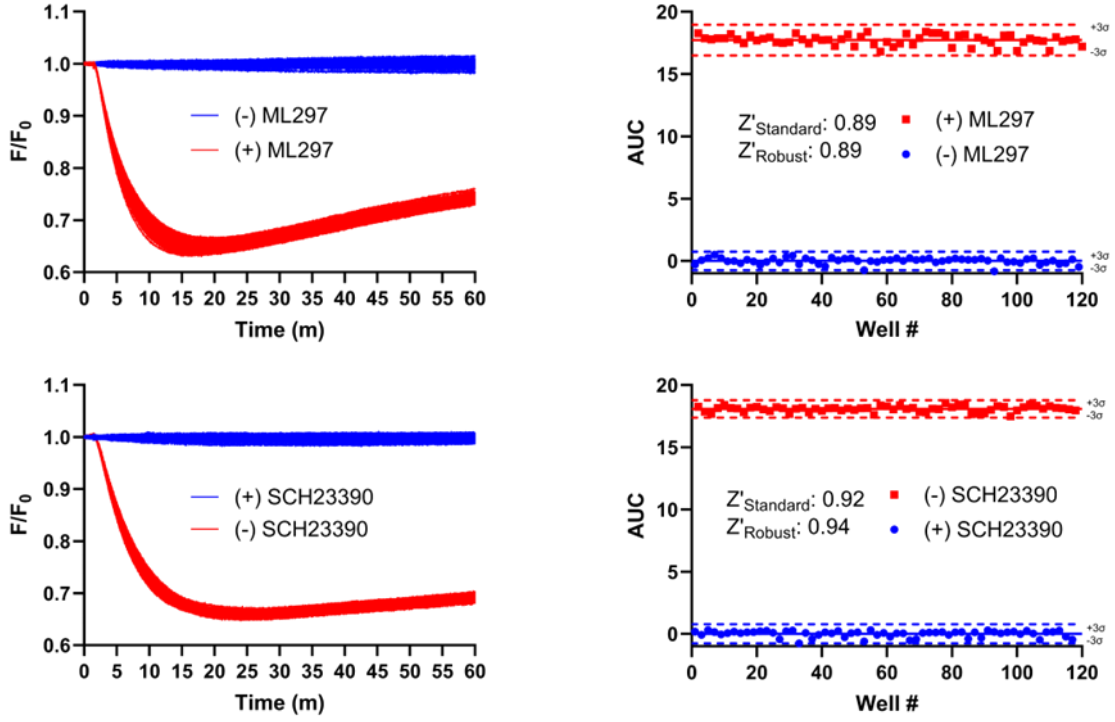

Figure 8. Z' Determination in K_{ir}3.1/3.2-Expressing HEK293 Cells. Baseline-normalized kinetic fluorescence data collected using Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm). ±SCH23390, a selective GIRK Channel inhibitor, was pre-incubated for 15 minutes at a concentration of 100 µM before the start of the assay. ±ML297, a potent activator of GIRK Channels, was added at 60 seconds at a concentration of 10 µM. The AUC was calculated between the curve and y=1. The Z' factor plots show a Z' above 0.8 for both ML297 activation and SCH23390 inhibition of K_{ir}3.1/3.2 (n = 60).

Example Results (Continued)

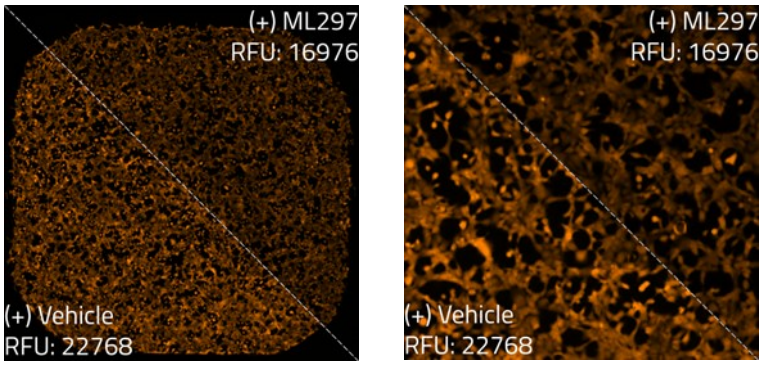

Figure 9. ML297 Activation of GIRK Channels in HEK293 Cells Expressing Kir3.1/3.2. HEK293 cells stably expressing K_{ir}3.1/3.2 were plated in a 384-well format, incubated with Dye Loading Solution, and treated with Stimulus Solution containing ML297 or vehicle for 1 hour, resulting in a final well concentration of 10 µM ML297. Whole-well images were acquired using a Cytation 5 imager with a 4X objective, RFP filter (Ex: 531/40 nm, Em: 593/40 nm).

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 GIRK 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