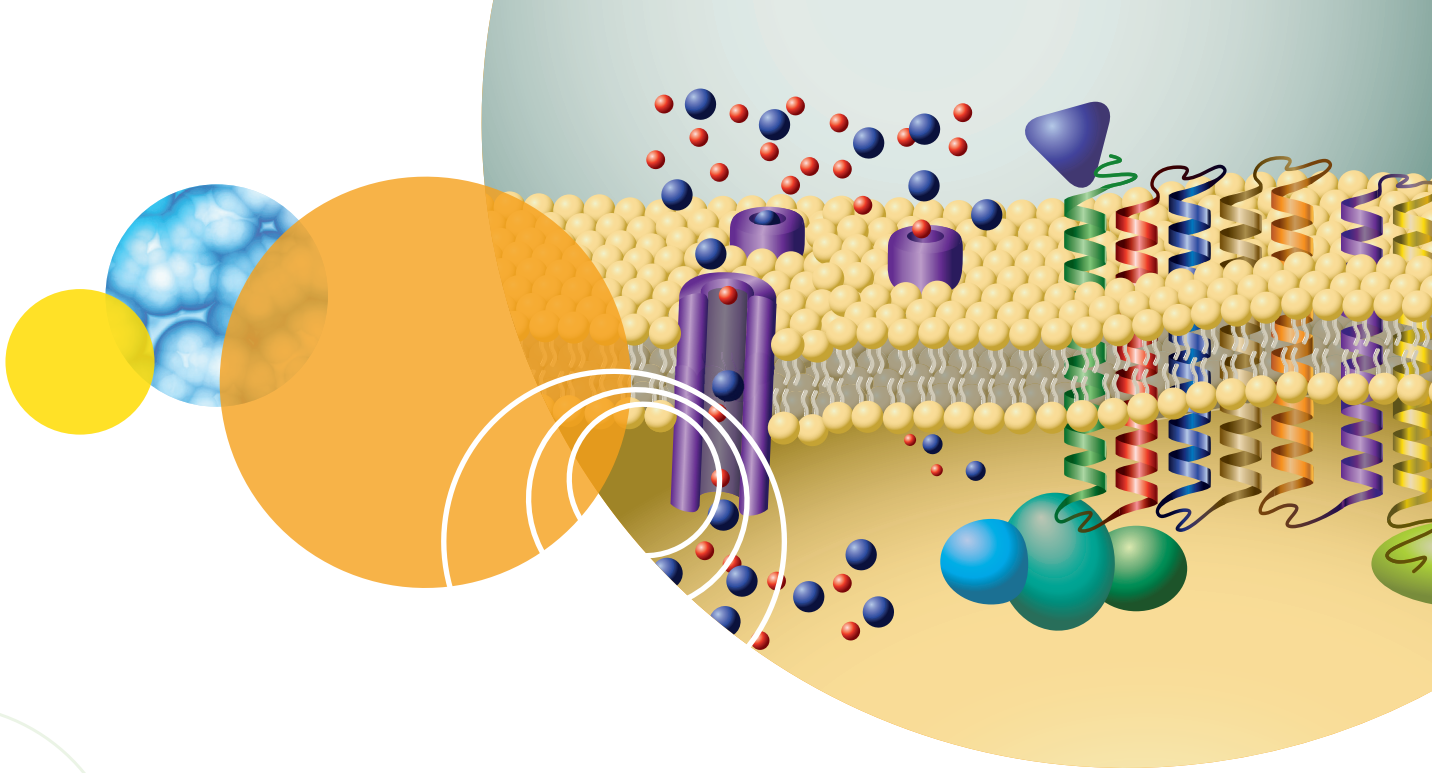

Identify Targets Like a Pro

Solutions for identifying early leads against GPCRs and ion channel targets

Our FLIPR Tetra® High-Throughput Cellular Screening System is fast, reliable, and remarkably easy to configure. The system is optimized for use with both fluorescence and luminescence, and adapts readily to your assay format with user-changeable 96-, 384-, and 1536-well heads.

eBook contents

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Comparison of intracellular calcium measurements

G protein-coupled receptors (GPCRs) play an important role in cell signaling. When the receptor is activated by a ligand, receptor conformation is changed, triggering G-protein activation inside the cell. An active G protein has the potential to induce various cascades of intracellular messengers including calcium. The FLIPR Tetra System performs high-throughput, functional cell-based assays and is the system of choice in drug discovery for evaluating changes in intracellular calcium detected through use of fluorescent calcium-sensitive reporter dyes. Here we provide a basic protocol for performing a calcium mobilization assay on the FLIPR Tetra System using the FLIPR® Calcium Assay Kit, a homogeneous, fast and reliable fluorescence assay.

- Perform high-throughput, functional cell-based assays
- Quickly evaluate changes in intracellular calcium
- Homogeneous, fast and reliable fluorescence assay

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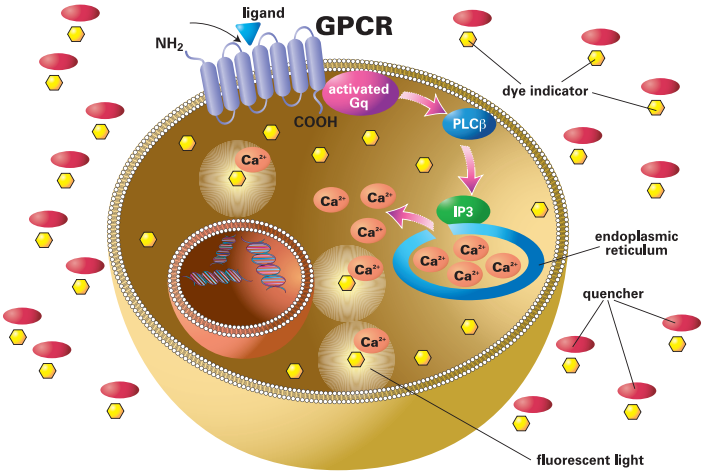
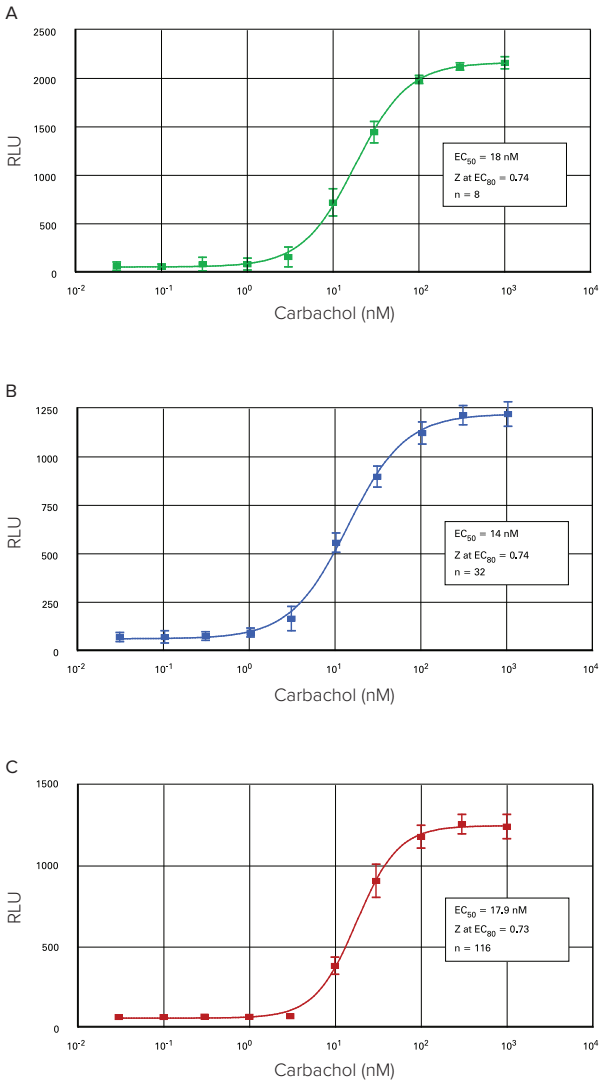


Calcium mobilization assay principle. Increase in cytosolic calcium can be detected by fluorescence measurement using calcium-sensitive indicator dyes.



Carbachol dose-response curves. Calcium mobilization by carbachol in CHO M1WT3 cells in three formats generated with the FLIPR Calcium Assay Kit on the FLIPR Tetra System. **(A)** 96-well; **(B)** 384-well; **(C)** 1536-well.

Comparison of a novel calcium assay to fluorescence-based calcium flux assays

Cell-based calcium flux assays are widely used in high-throughput screening (HTS) for identification of GPCR agonists and antagonists as well as other applications such as cardiac beating assays. Here we introduce a reagent system utilizing a novel calcium-sensitive ionophore that has a larger signal window with low background while maintaining Z' factors.

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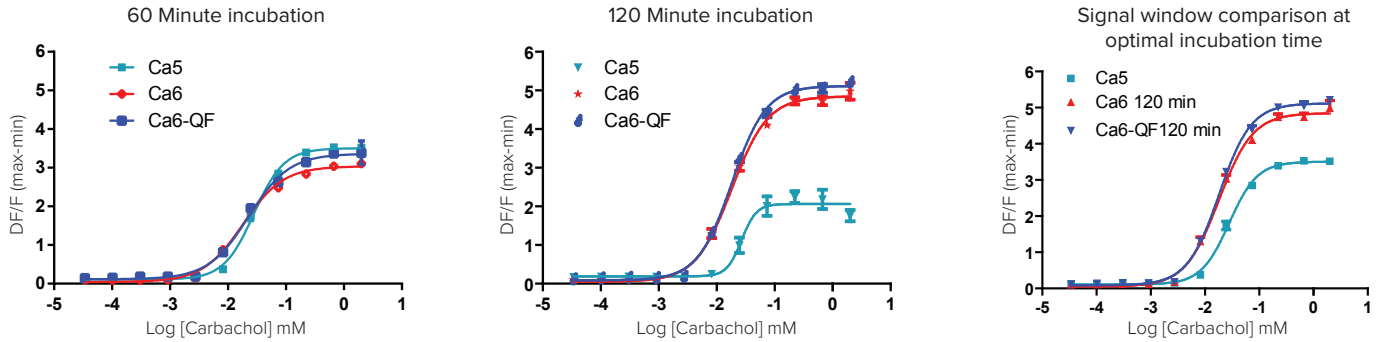
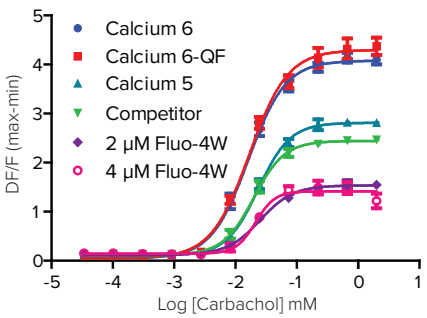
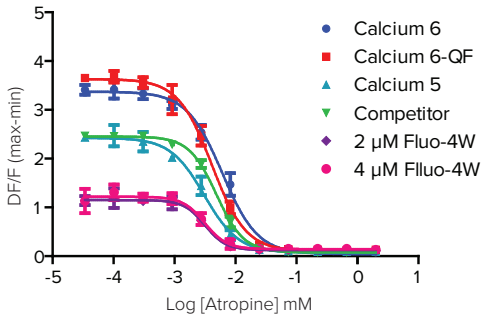
- Enable low signal screens, including endogenous, primary or stem cell targets
- Lower background fluorescence significantly with masking technology
- Increase signal-to-noise ratio without removing growth media



Optimization of assay incubation time shows that both Calcium 6 and Calcium 6-QF assays benefited from a 2 hour incubation to achieve maximum signal window due to larger molecule size. Calcium 5 Kit and competitor dyes were incubated at their optimal incubation time of one hour. All assays were run with CHO-M1 cells in buffer. EC_{50} values were comparable to historical values (data not shown) and Z @ EC_{80} values were > 0.8.



	Ca 6	Ca6-QF	Ca 5	Competition	2 µM Fluo4	4 µM Fluo-4
EC_{50}(nM)	16	17	23	20	25	21
Z at EC_{80}	0.88	0.84	0.89	0.85	0.86	0.71



	Ca 6	Ca6-QF	Ca 5	Competition	2 µM Fluo-4	4 µM Fluo-4
IC_{50}(nM)	6	4	3	5	3	3
Z at IC_{80}	0.77	0.76	0.7	0.83	0.53	0.1

Comparison of Calcium 6 kits to other calcium flux assays. Each assay was incubated at optimal time for dye loading. Fluo-4 wash assay had the smallest signal window due to greater cell manipulation and extracellular fluorescence background. Both Calcium 6 and Calcium 6-QF kits provided the highest signal windows compared to the other kits or Fluo-4 Wash. EC_{50} values were preserved across all assays and Calcium 6 Kits showed Z at EC_{80} > 0.84 in the agonist assay and Z at IC_{80} > 0.76 in the antagonist assay.

Calcium release-activated channel (CRAC) assays

Calcium release-activated calcium (CRAC) channels play an important role in intracellular Ca2+ homeostasis. Like other store-operated calcium (SOC) channels, currents through the CRAC channel (I_{CRAC}) are activated by depletion of calcium in the endoplasmic reticulum (ER) and serve the purpose of slowly replenishing the ER with Ca^{2+}. CRAC channels are considered key for the activation of immune cells. Abnormalities in I_{CRAC} have been associated with certain primary immunodeficiencies, acute pancreatitis and abnormal cell proliferation. Hence the development of a reliable and sensitive CRAC channel assay may lead to the detection of novel therapeutic agents aimed at treating these human disorders.

Here we compare ratiometric Fura-2 AM, non-ratiometric Fluo-4 NW and the FLIPR® Calcium 6 and Calcium 6-QF Assay Kits in two CRAC channel assays, using both RBL and Jurkat cells.

- Background fluorescence is reduced by masking technology
- Dye formulation delivers larger signal window due to enhanced retention of dye within the cell
- Calcium 6-QF formulation is a flexible option for quench-sensitive targets or multiplexing applications

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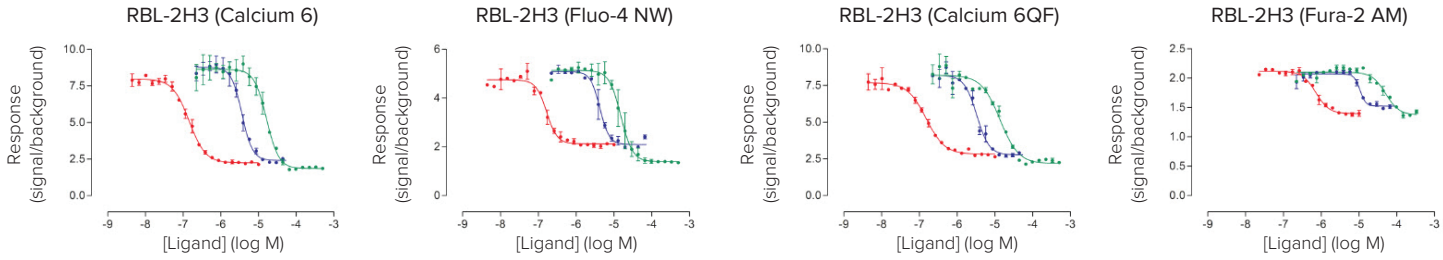
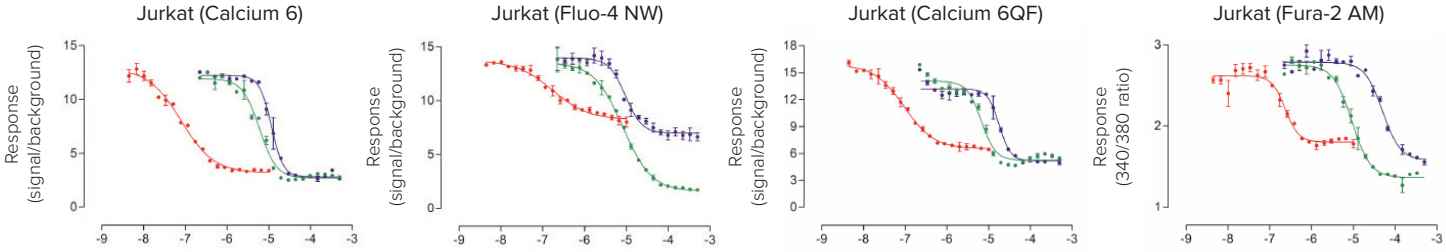


CRAC channel inhibitor assay in adherent RBL-2H3 cells. Dyes were loaded according to protocols outlined in the methods section and inhibition curves obtained with YM 58483 (●), Econazole (●) and 2-APB (●). The rank order of inhibitor potency in this assay was YM 58483 » 2-APB > Econazole.



CRAC channel inhibitor assay in suspension Jurkat cells. Dyes were loaded according to protocols outlined in the methods section and inhibition curves obtained with YM 58483 (●), Econazole (●) and 2-APB (●). The rank order of inhibitor potency in this assay was YM 58483 » Econazole ≥ 2-APB.

Potassium ion channel assay for high-throughput screening

Potassium channels are responsible for a variety of cellular functions including the maintenance and regulation of membrane potential, secretion of salts, hormones, and neurotransmitters. The dysfunction of potassium channels has been associated with many human diseases. 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. Challenges exist in measuring K^+ ion channel activity in a high-throughput format. A widely adopted technique is to use the fluorometric method where the binding of thallium to thallium-sensitive fluorescent dyes is utilized as a surrogate measurement of potassium channel activity.

Here we demonstrate how the FLIPR® Potassium Assay Kit analyzes potassium ion channel activities on the FLIPR Tetra System.

- Functional measurement of K^+ channel activities
- Homogeneous no-wash protocol enhances ease-of-use and reduces total assay time
- Reduced well-to-well variation and improved data quality compared to non-homogeneous formats

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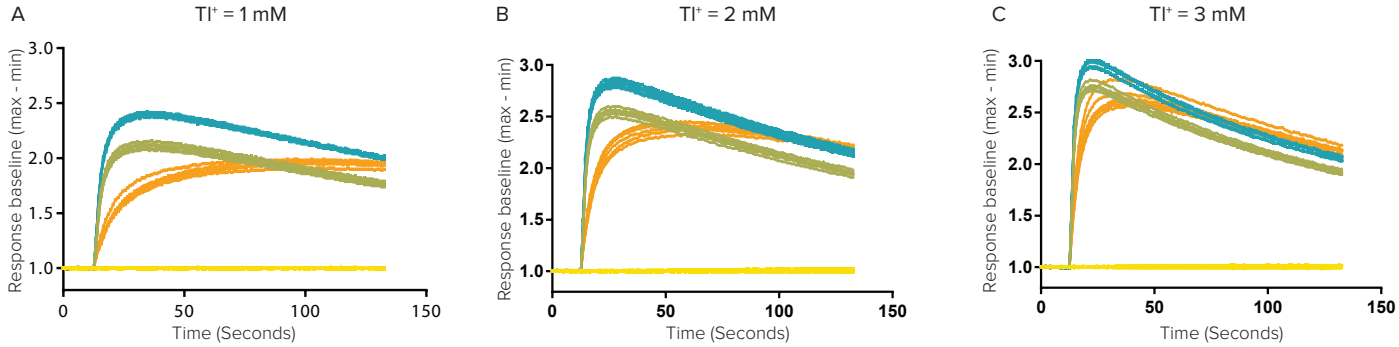
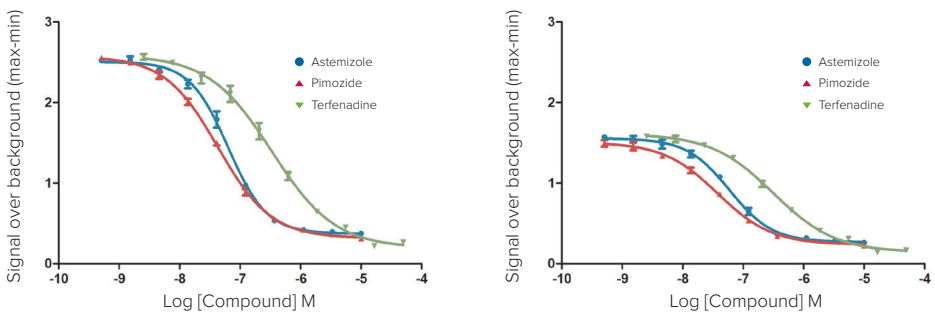


K_V1.3 Channel Activity: Assay optimization with Tl^+/K^+ Titration. K_V1.3 channel activity was measured with different K^+ and Tl^+ concentrations for optimal assay performance. [K^+] = 10 mM (—), 20 mM (—), or 30 mM (—) was used as stimulus in the presence of 1 mM Tl^+ **(A)**, 2 mM Tl^+ **(B)** and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



hERG channel pharmacology. IC_{50} determination of 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.

Characterization of hERG channel blockers using the FLIPR Potassium Assay Kit

Drug-induced inhibition of the human ether-à-go-go-related gene (hERG) ion channel has been related to the susceptibility of patients to potentially fatal ventricular tachyarrhythmia, torsade de pointes. In recent years, a number of FDA-approved drugs were withdrawn from the market due to their off-target effect on hERG. As a result, there has been an increasing need for identifying compounds that block the hERG channel at earlier stages in the drug discovery process. Here we present the utility of the FLIPR Potassium Assay Kit on the FLIPR Tetra System to investigate hERG compound activity.

- Functional measurement of K^+ channel activity in a cell-based assay
- Homogenous no-wash protocol reduces well-to-well variation and simplifies the workflow
- Expanded signal window compared to non-homogenous assay

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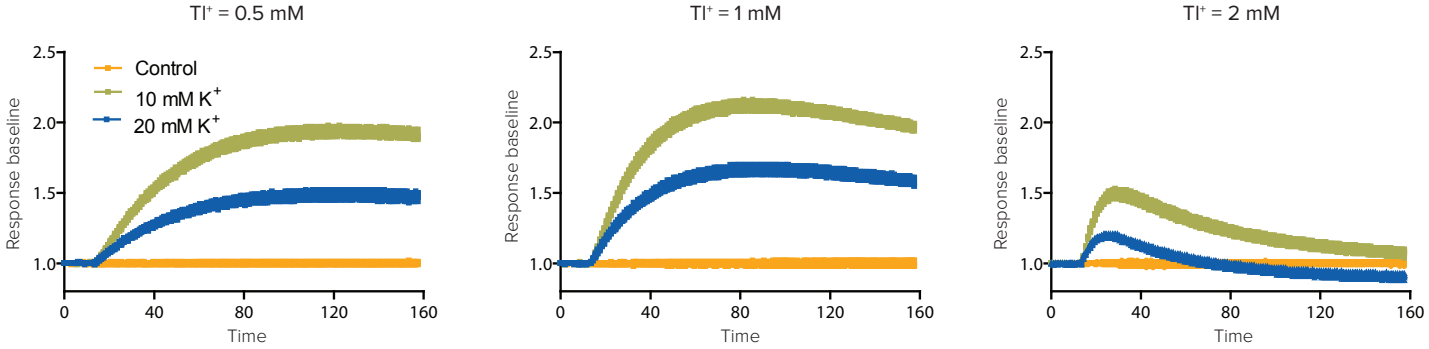
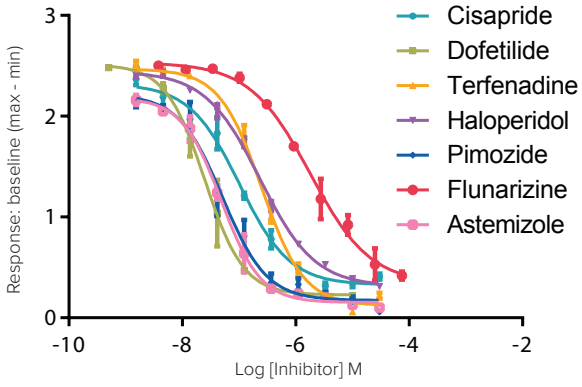
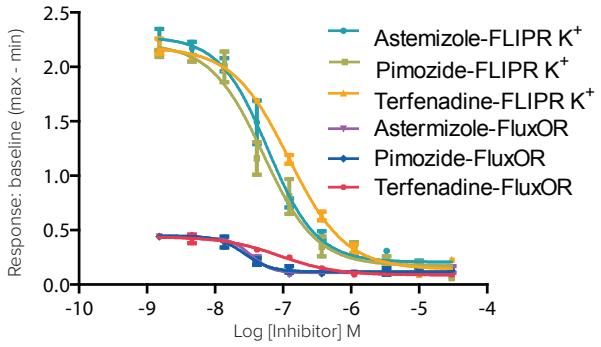


Optimization of hERG channel stimulant. Cells were incubated with dye and the stimulant buffers were added during detection on the FLIPR Tetra System. The concentration-dependent response of signal was characterized under different conditions. Optimal signal was obtained from the combination of 1 mM Tl^+ and 10 mM K^+ (final concentration) stimulant buffer diluted in chloride-free buffer.



Concentration-dependent inhibition of hERG channel by reference compounds.



Comparison of FLIPR Potassium Assay Kit results to a competitor kit.

Homogeneous solution for GPCR assays

Cell-based assays have become an indispensable method for screening and compound profiling in the early drug discovery process. To date, such assays have proven to be some of the most reliable and reproducible methods in receptor characterization studies, primary screening campaigns and compound profiling programs. For G_q-coupled GPCR targets specifically, homogeneous fluorescent calcium flux assays with masking technology are the methodology of choice.

Combining a novel fluorophore and proven masking technology, the FLIPR Calcium 5 Assay Kit delivers reliable pharmacology, a larger signal window, and improved assay performance. With the FLIPR Calcium 5 Assay Kit and the FLIPR Tetra System, consistent screening of a variety of receptors and targets, especially those with small calcium signal responses, can be obtained in an easy-to-use, homogeneous format.

- Superior signal-to-noise ratio over competitive dyes and kits
- Robust data, with Z' factor > 0.9
- Low well-to-well variation, even with frozen cells
- True homogeneous protocol, mix-and-read

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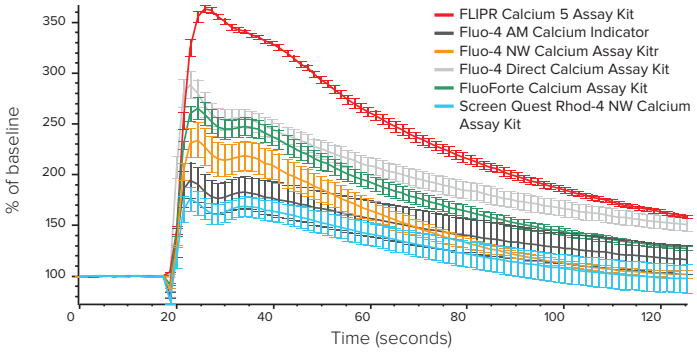
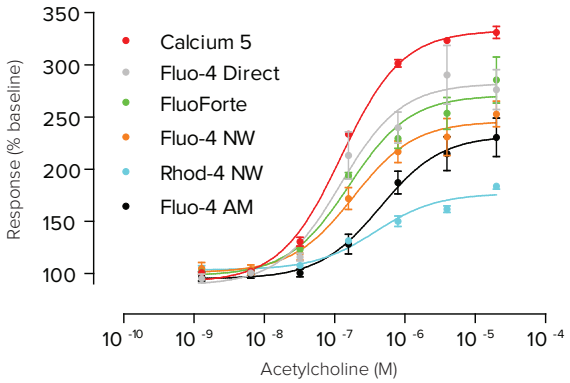
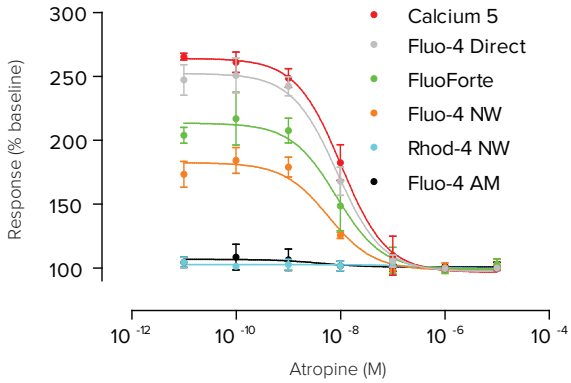


Kinetic traces from the FLIPR Tetra System. Representative signal traces on the FLIPR Tetra System for acetylcholine induced agonism of the endogenous muscarinic M3-receptor in "assay ready" 1321N1 cells compared using different calcium assay kits.



Agonism: comparison of calcium assay signal window. Comparison of the fluorescent signal in "assay ready" 1321N1 cells during acetylcholine stimulation of the endogenous muscarinic M3-receptor. This illustrates the enhanced signal-to-background ratio obtained by the FLIPR Calcium 5 Assay Kit with proven masking technology.



Antagonism: comparison of calcium assay signal window. Atropine inhibition of calcium flux in response to an EC_{80} challenge of acetylcholine in "assay ready" 1321N1 cells, evaluated with six different calcium reagents on the FLIPR Tetra System.

Live cell G_i- and G_s-coupled GPCR second messenger signaling

Detection of G_i- and G_s-coupled GPCR second messenger signal activity has been traditionally accomplished using assays such as radioactive binding or endpoint cAMP assays that require cell lysis. Such assays measure activity at a single time point in the cellular response and do not provide kinetic information. Another option utilizes forced-coupling of G_i- and G_s-GPCRs to Gα16 followed by fluorescence detection of calcium flux upon agonist receptor activation. Again, this assay is sub-optimal as it does not signal through the biorelevant cAMP pathway.

Here we demonstrate endogenous receptor activity in CHO-K1 and HEK-293 cell lines stably expressing the GloSensor™ plasmid using the FLIPR Tetra System.

- GloSensor cAMP Assay is demonstrated on the FLIPR Tetra System as a live cell HTS screening application for G_i- and G_s-coupled GPCRs
- Use of the FLIPR Tetra System with GloSensor cAMP Assay enables kinetic measurement of G_i- and G_s-coupled receptor signaling not possible using endpoint assays on standard plate readers

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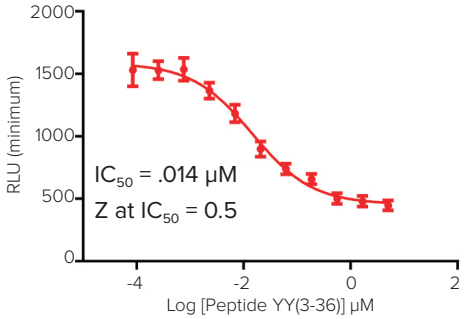
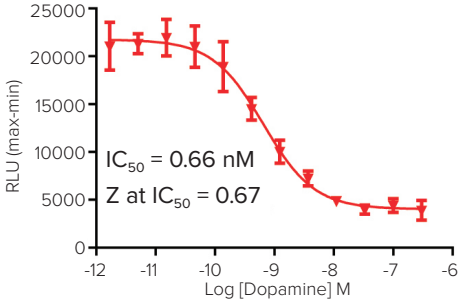
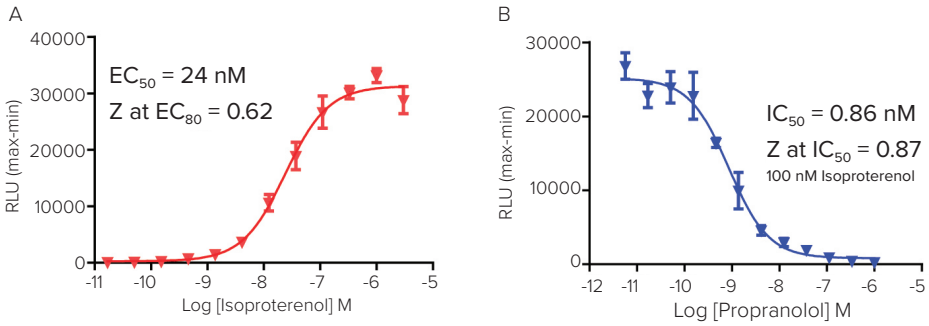


G_i-coupled agonsim. G_i-coupled GPCR receptor agonism results in a reduction in signal correlated with reduction in cAMP inside the cell. Baseline increase in cAMP activity was induced by the addition of forskolin. Using stable P2Y receptor in CHO-K1 cells, we compared results upon addition of forskolin either before or after the agonist. 10 µM forskolin addition followed 15 minutes later by addition of agonist Peptide YY(3-36) on the FLIPR Tetra System.



HEK-293 cells over expressing G_i-coupled D4 receptor. HEK-293 cells over expressing G_i-coupled dopamine D4 receptor from Multispan, Inc. were transiently transfectd with GloSensor cAMP-22F plasmid. Ligand was added on-line to the wells, followed by 5 minute incubation. Continuing the assay, FLIPR Tetra System added 10 µM forskolin to stimulate cAMP production in the cell. Inhibition of forskolin mediated cAMP production by Dopamine.



G_s-coupled GPCR agonist and antagonist. Transient transfection of GloSensor cAMP-22F and endogenous G_s-coupled cAMP response in HEK 293 cells. **(A)** Response to isoproterenol and **(B)** inhibition of the response to 100 nM isoproterenol by propranolol. Results are comparable to those obtained from the experiment performed with the stable GloSensor HEK-22F cell line.

Compound effects upon calcium transients in Cor.4U human iPSC cardiomyocytes

There is a growing need for highly predictive *in vitro* cardiotoxicity assays that use biologically relevant cell-based models and are suitable for high-throughput screening. iPS cell-derived cardiomyocytes are especially attractive cell models because they represent gene expression profiles as well as phenotypic characteristics similar to native cardiac cells.

The calcium sensitive dye in the EarlyTox™ Cardiotoxicity Kit makes it possible to evaluate concentration-dependent modulation of calcium peak frequency and illustrate oscillation patterns in Axiogenesis Cor.4U® iPS cell-derived cardiomyocytes using the FLIPR Tetra System.

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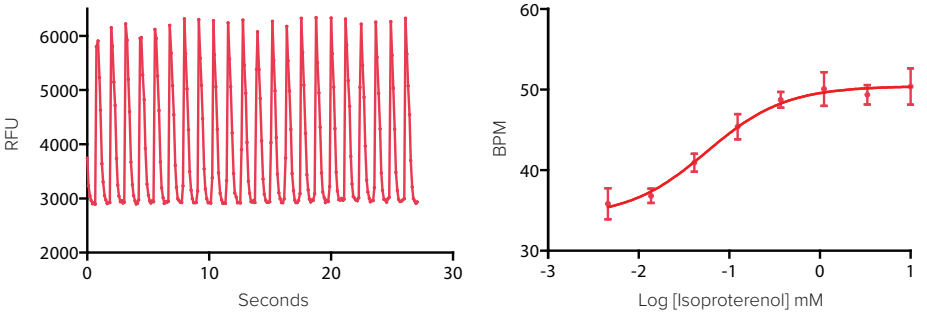
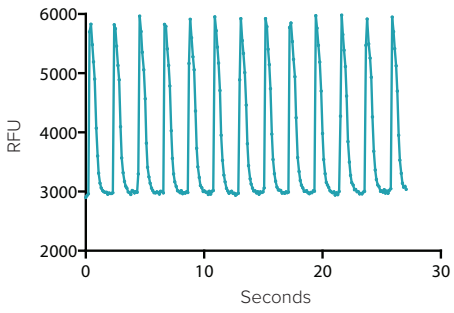
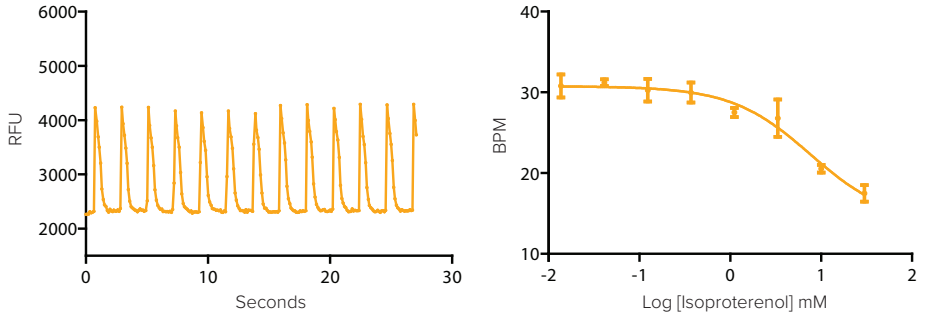
- Evaluate compound toxicity and efficacy earlier in the drug discovery process
- Analyze cardiotoxicity profiles in a biorelevant system
- Scale assay size to meet throughput requirements



Isoproterenol β adrenergic agonist. Left: Calcium signal oscillation in response to 0.37 µM isoproterenol. **Right:** Three minutes post compound addition, increase in isoproterenol concentration increases beat frequency of iPS cell-derived cardiomyocytes from approximately 36 to 50 BPM.



Calcium signal oscillation. In the experimental control, calcium signal oscillation reflects changes in cytoplasmic calcium concentration in Cor.4U iPS cell-derived cardiomyocytes measured with the EarlyTox Cardiotoxicity Kit on the FLIPR Tetra System.



Propranolol, a β adrenergic antagonist slows beat frequency in iPS cell-derived cardiomyocytes. Left: Beat pattern in response to 3.3 µM propranolol. **Right:** BPM at 3 minutes post compound addition slows from approximately 31 to 17 with increase in compound concentration.

High throughput cardiotoxicity assays using stem cell-derived cardiomyocytes

The development of highly predictive *in vitro* assays suitable for high throughput screening is critical to improve the inefficiencies and high costs associated with cardiac safety compound failure. Traditional methods for characterizing cardiotoxic compounds are labor-intensive and slow.

With ScreenWorks® Peak Pro Software running on the FLIPR Tetra System, you can quickly and easily characterize cardiotoxic compounds using stem cell-derived cardiomyocytes. Human cardiomyocytes derived from stem cell sources can greatly accelerate the development of new chemical entities and improve drug safety by offering more biologically relevant cell-based models than those presently available.

- Earlier prediction of compound toxicity and efficacy
- Robust, high throughput, biologically relevant assays
- Fast, simplified data analysis for compound prioritization

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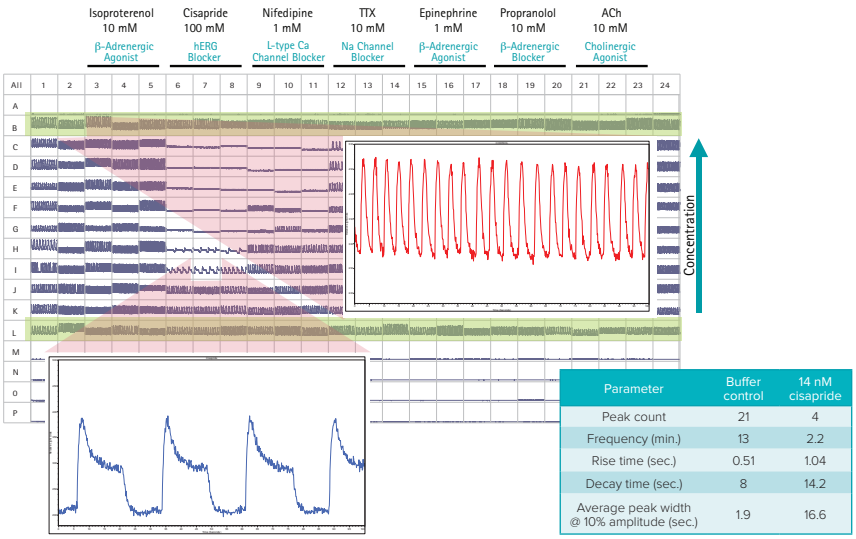
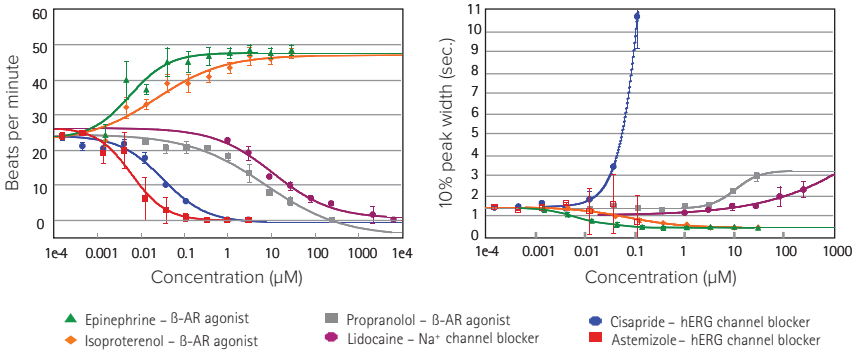


Parameter	Buffer control	14 nM cisapride
Peak count	21	4
Frequency (min.)	13	2.2
Rise time (sec.)	0.51	1.04
Decay time (sec.)	8	14.2
Average peak width @ 10% amplitude (sec.)	1.9	16.6

Cardiomyocyte assay capabilities. Cardiomyocyte contraction parameters calculated by ScreenWorks Peak Pro Software for two different wells.



Effects of β-adrenergic receptor agonists/antagonists and ion channel blockers. Dose response of six different compounds as measured by the FLIPR Tetra System in Calcium 5 loaded iPSC derived cardiomyocytes. **Left:** Change in frequency of contractions with dose. **Right:** Change in average peak width with dose.

Optimization of $Na_V1.5$ channel assay

Voltage-gated ion channels are present in the excitable cell membranes of heart, skeletal muscle, brain and nerve cells. Blocking or modulating such channels can have a therapeutic effect, or may interfere with normal cell function. As a result, compounds that affect voltage-gated ion channels are important targets in drug discovery. Cardiac $Na_V1.5$ channels are classified as "Tetrodotoxin (TTX)-resistant." The pharmacological significance of the $Na_V1.5$ channel is that they are targets for the action of antiarrhythmic drugs and are also blocked by local anesthetics such as lidocaine.

FLIPR® Membrane Potential Assay Kits provide a rapid and reliable fluorescence-based method to detect changes in membrane potential brought about by compounds that modulate or block voltage-gated ion channels held open by veratridine. We offer two different no-wash formulations of FLIPR Membrane Potential Assay Kits: Blue and Red. Each kit uses a proprietary indicator dye, combined with different quencher to maximize cell line/ channel/compound applicability while eliminating causes of variability in the data.

- Maximize cell line/channel/ compound applicability with proprietary indicator dye
- Eliminate causes of data variability
- Detect bidirectional gradient changes to both variable and control conditions within a single experiment

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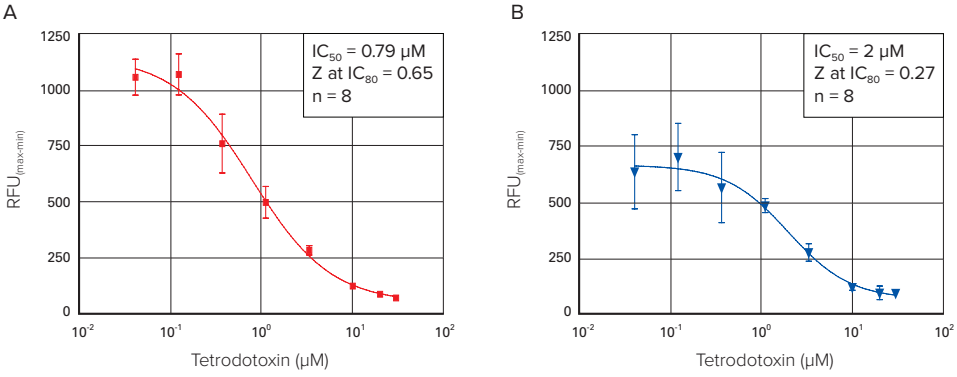
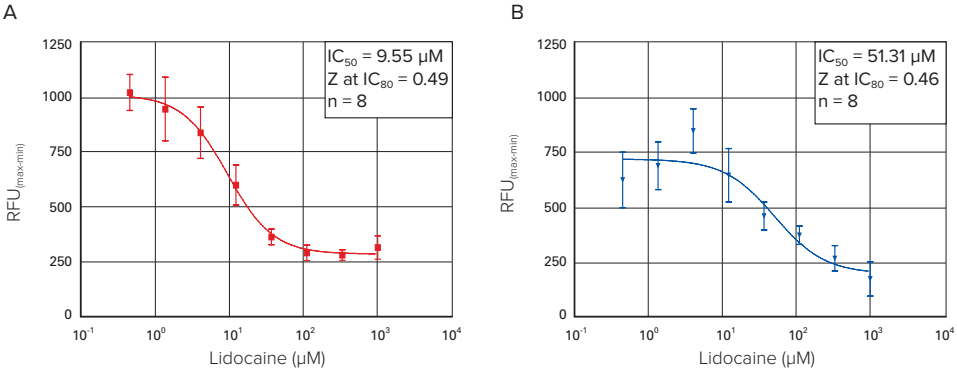


Modulation of $Na_V1.5$ Channel in CHL Cells by Tetrodotoxin. Comparison of tetrodotoxin IC_{50} curves. **(A)** FLIPR Membrane Potential Red Assay Kit. **(B)** FLIPR Membrane Potential Blue Assay Kit. A larger response with a better Z' factor is seen in FLIPR Membrane Potential Red Assay Kit.



Modulation of $Na_V1.5$ Channel in CHL Cells by Lidocaine. Comparison of lidocaine IC_{50} curves. The FLIPR Membrane Potential Red Assay Kit (A) results in a greater fluorescence change and a more sensitive response to lidocaine compared to the FLIPR Membrane Potential Blue Assay Kit (B).

Cryopreserved BacMam-transduced Aequorin cells

Aequorin is a photo-sensitive protein that emits luminescent light in response to calcium. Cryopreserved cells as reagents in Aequorin-based calcium flux assays decouples the tissue culture process from high-throughput screening while improving overall assay performance. The need for culturing cells in plates is eliminated.

Here we demonstrate the performance of cryopreserved BacMam-transduced Aequorin cells (provided by GSK) in 384- well and 1536-well formats. Combined with the FLIPR Tetra System equipped with Aequorin options, cryopreserved cells are a powerful tool in the identification of lead compounds in drug discovery.

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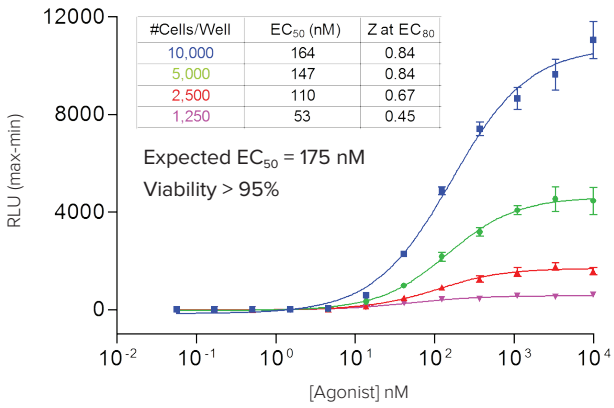
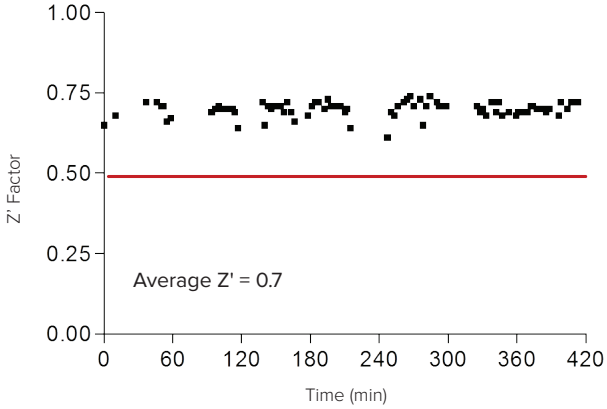
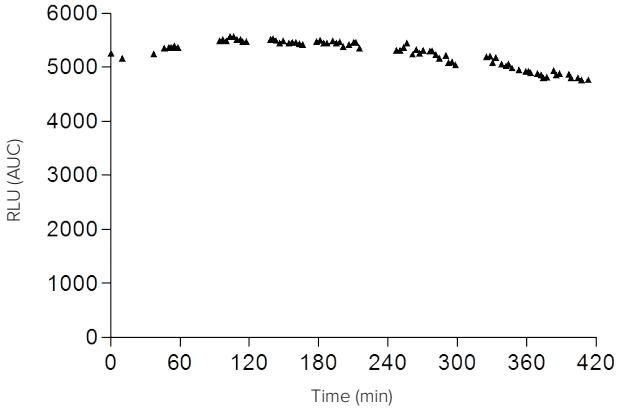
- These cell lines can be used to produce robust assay results in both 384-well and 1536-well formats on the FLIPR Tetra System with Aequorin options
- Aequorin-based suspension assays with frozen cells demonstrate both instrument and cell performance during extended assays without significant shift in EC_{50} or Z' factor
- The EC_{50} values remain within one half log of expected results and there is little reduction in signal intensity over time



Cell Titration and CRC Assay measured with FLIPR Tetra System. In a 384-well plate, frozen Bacmam Transduced Aequorin Cells were titrated against a CRC of target agonist. In this assay, cells were pre-plated in suspension prior to addition of compound.



Z' factor over time during BacMam Aequorin Suspension Cell Assays. Recording of whole plate Z' factor during extended plate screening assays. 384-well plates contained 22 columns of EC_{80} reference compound and 2 columns of negative controls. Average Z' factor = 0.7.



Change in signal over time during BacMam Aequorin Suspension Cell Assays. Change in RLU over time during six hour 384-well suspension cell experiment. Cells were added at 2,500/well.

Optional FLIPR Tetra System assay configurations

We offer a broad range of user-installable optics for the FLIPR Tetra System to enable the application flexibility you require to support your research and screening. You can easily change the LEDs and filters on the system in just a few minutes.

Target assay application	Ex LED (nm)	Em filter (nm)
Fluorescence		
Fura-2	335-345 and 380-390	475-535
MQAE	360-380	400-460
Voltage Sensor Probe (VSP)	390-420	440-480
Tango GPCR assay system (FRET)	390-420	440-480, 515-575
CFP; eCFP/YFP	420-455	475-535
FLIPR Calcium Assay	470-495	515-575
Calcium (Fluo-4), Calcium (Fluo-8)	470-495	515-575
Calcium (Fluo-3)	495-505	526-586
JC-1	495-505	565-625
FLIPR Membrane Potential Assay	510-545	565-625
Rhodamine-2, Rhodamine-4	510-545	565-625
Alexa 633 and Bodipy	610-626	646-706
Luminescence		
Aequorin	Luminescence	
GloSensor	Luminescence	
BRET 2	Luminescence	440-480, 526-586

Additional dyes and applications may be addressed. Ask us for more information about your applications of interest. Custom filter holders are available to work with additional emission filters for your applications.

Ordering information		
Product	**Description**	**Part number**
FLIPR Tetra System	High-Throughput Cellular Screening System	FLIPR
FLIPR Tetra Pipette Tips	Black, non-sterile, 96-well 50 racks/case	9000-0762
FLIPR Tetra Pipette Tips	Clear, non-sterile, 96-well 50 racks/case	9000-0761
FLIPR Tetra Pipette Tips	Black, non-sterile, 384-well 50 racks/case	9000-0764
FLIPR Tetra Pipette Tips	Clear, non-sterile, 384-well 50 racks/case	9000-0763

Download collateral

- **Brochure**
- **Data Sheet**

View related webinars

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- **Developing an *in vitro* Tl^+ flux assay for potassium channel agonist identification**
- **Novel *in vitro* assay tools for cardiac toxicity and discovery**
- **Optogenetics and the FLIPR: how to use light to set up a $Ca_v1.3$ high-throughput screening assay**

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