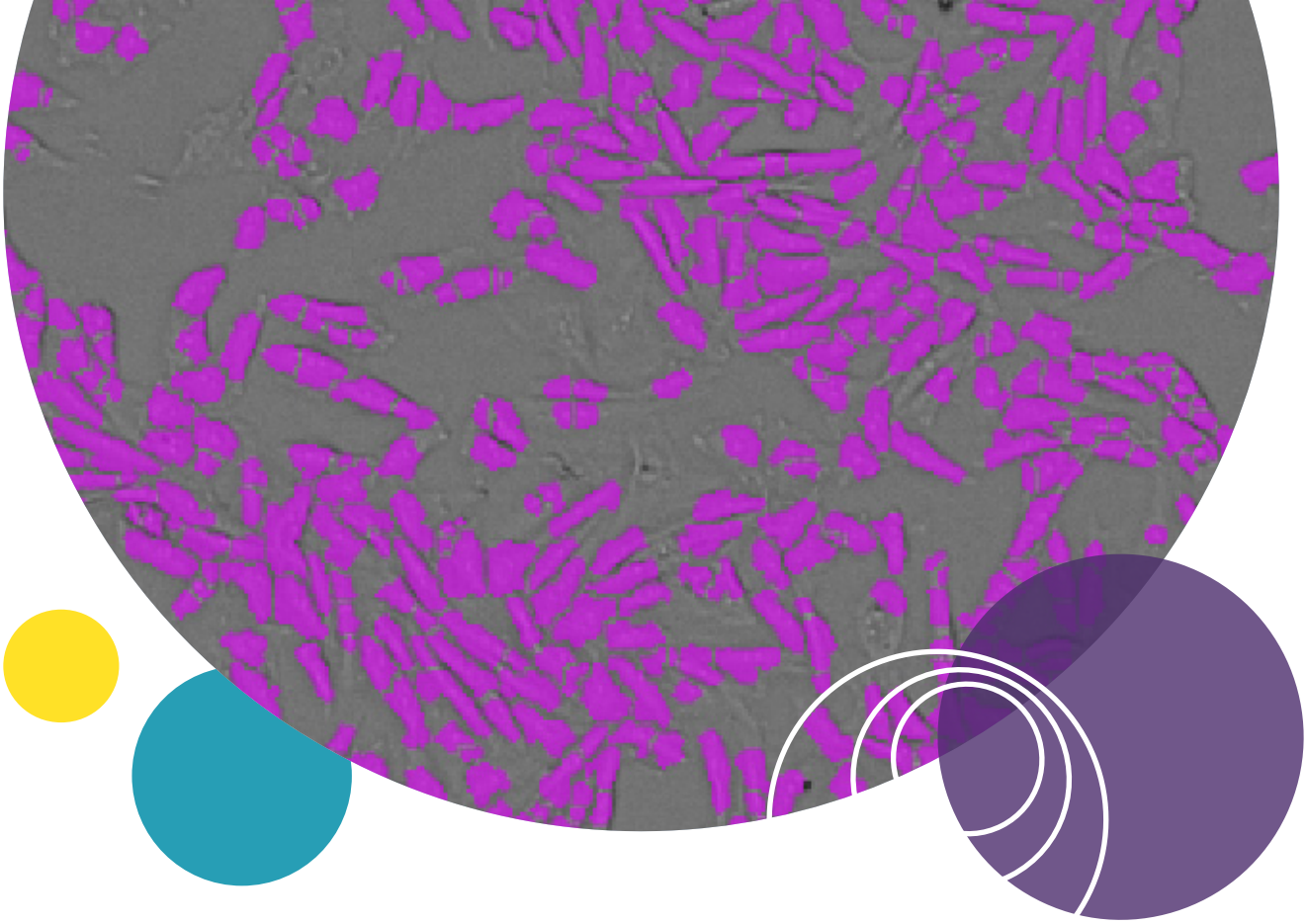

Fast track cell-based assays like a pro

Our FlexStation® 3 Multi-Mode Microplate Reader is a multi-detection platform that increases liquid handling throughput and flexibility for biochemical- and cell-based kinetic assays. With advanced dual optical systems operating above and below your microplates, it measures absorbance, fluorescence intensity, fluorescence polarization, luminescence, and time-resolved fluorescence. Utilizing integrated fluidics, the reader adds your assay reagents from a 96- or 384-well source plate requiring you give up nothing in assay design. With five optical detection modes and programmable plate-to-plate liquid handling, you'll create fast new assays.

eBook contents

Development of a platelet calcium flux assay 2

Characterization of hERG channel blockers 3

Monitoring receptor mediated changes in $[Ca^{2+}]_i$ 4

Dual-Luciferase Reporter Assay detection 5

Comparison of Photina luminescent calcium mobilization assays 6

Measurement of calcium signaling 7

Optimization of a muscarinic M_3-receptor assay 8

System configurations 9

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MOLECULAR DEVICES

Development of a platelet calcium flux assay

Platelets are small, anucleate blood cells that mediate haemostasis by aggregating at sites of blood vessel injury to form a thrombus (or clot) that limits blood loss. When platelets respond to vessel damage inappropriately, this can lead to thrombotic disease such as heart attacks and ischaemic stroke.

Intracellular Ca^{2+} is a master regulator of platelet function and has a critical role in thrombotic disease, but can be difficult to measure in fragile human platelet. The FlexStation 3 reader assay described here enables accurate and rapid measurements of agonist EC_{50} and antagonist IC_{50} estimates, and could support medium throughput screening of novel drugs using this primary human tissue.

- Real-time kinetic measurement of intracellular Ca^{2+} changes in platelets provides information- rich data
- Ability to miniaturize assay from cuvettes to ½ area well plates decreases platelet and compound usage
- On-board pipettor improves well-to-well reproducibility and assay robustness on FlexStation 3 reader

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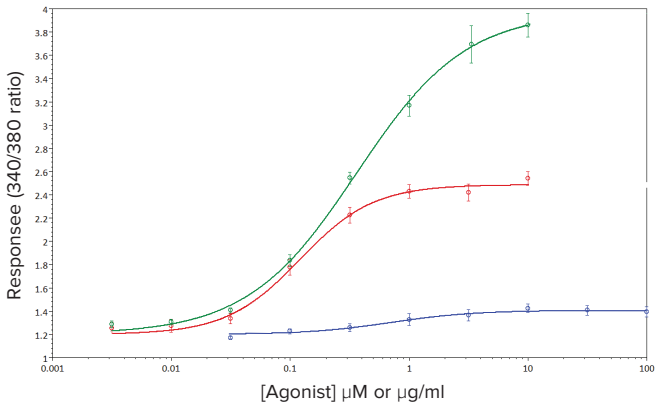
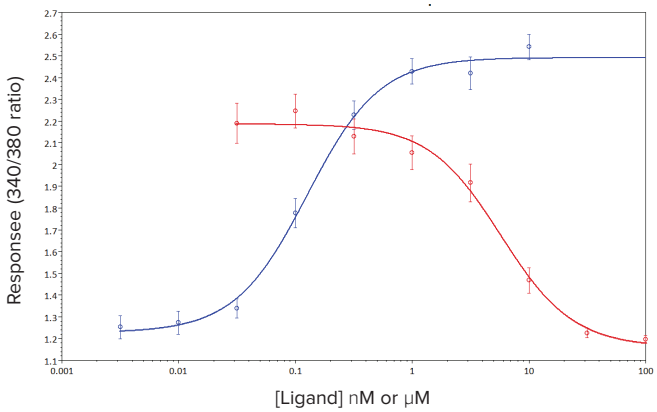
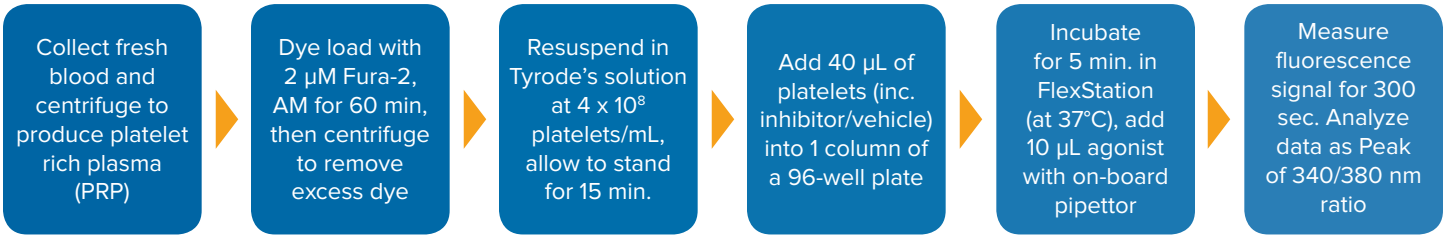


Concentration-response curves to ADP (○), U46619 (○) and CRP-XL (○) in Fura-2, AM-loaded human platelets. Data are mean values ± sem, n ≥ 10 from 5 independent experiments.



Mean concentration-effect curves for U46619 (○), and U46619 (EC_{80} concentration) in the presence of 8 different concentrations of GR32181B (○). Data are mean values ± sem, n ≥ 10 from five independent experiments.



FlexStation 3 reader platelet Ca^{2+} assay workflow using Fura-2, AM.

Characterization of hERG channel blockers

Drug-induced inhibition of the human ether-à-go-go-related gene (hERG) ion channel has been related to the susceptibility of patients to a 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 our FLIPR® Potassium Assay Kit on the FlexStation 3 reader 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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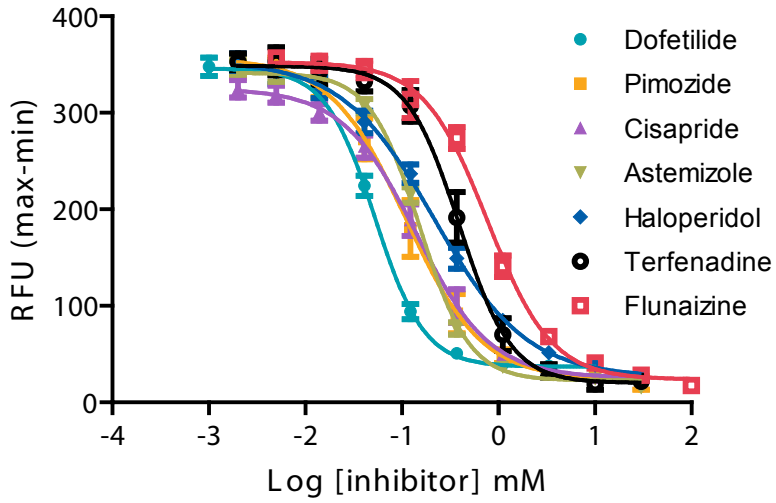
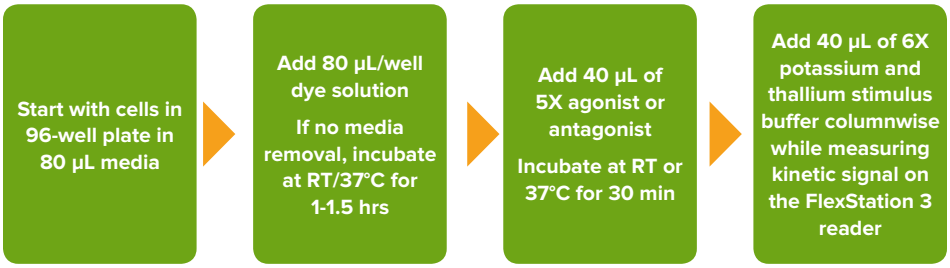
Assay Kits Compatibility Table



Concentration response curves of representative compounds that block hERG channel activities.
Data were collected from an assay performed on the FlexStation 3 reader.



FLIPR Potassium Assay Kit workflow.

Monitoring receptor mediated changes in $[Ca^{2+}]_i$

Calcium (Ca^{2+}) is the most common signal transduction element in cells ranging from bacteria to specialized neurons. Measurement of changes in the concentration of intracellular cations, for example Ca^{2+}, is important in understanding the mechanisms of many cellular processes. The FlexStation 3 reader integrates sensitive optics, fluid transfer and temperature control making it ideal for kinetic, cell-based fluorometric assays, such as the measurement of intracellular calcium, membrane potential and intracellular pH.

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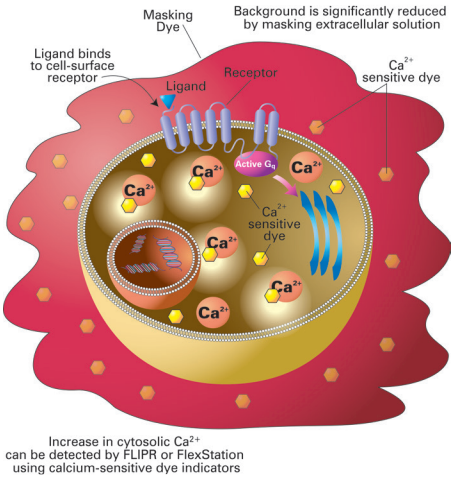
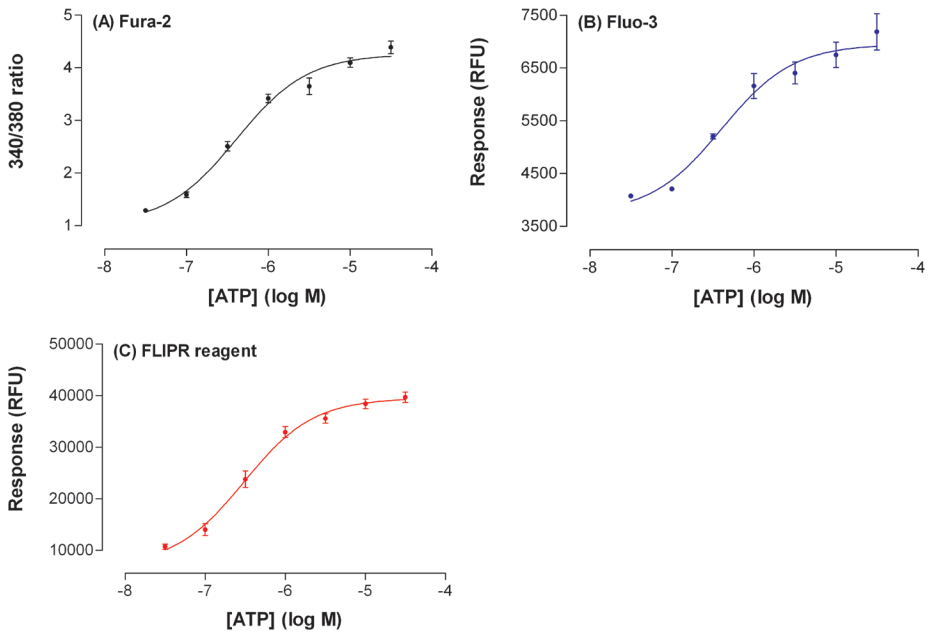
Assay Kits Compatibility Table

- Versatile reader for kinetic, cell-based fluorometric assays such as the measurement of intracellular calcium, membrane potential, and intracellular pH
- Reliable, reproducible data for calcium mobilization assays used to monitor Gq-coupled GPCRs
- Masking technology results in a five-fold increase in signal



Principle of the FLIPR calcium assay. The FLIPR Calcium Assay Kits include a calcium-sensitive dye that is taken into the cytoplasm of the cell during the incubation period. The cell-impermeant masking dye remains outside the cell and blocks background fluorescence. Upon ligand binding to the receptor, calcium is released into the cytoplasm of the cell. The dye binds to the intracellular calcium and an increase in fluorescence is measured.



ATP increase with fluorescent signal. Plot of ATP concentration (x-axis) against the increase in fluorescent signal (y-axis). Data is mean ± s.e. mean.

Dual-Luciferase Reporter Assay detection

Reporter-gene assays are useful tools in the study of eukaryotic gene expression. Dual-Luciferase Reporter Assay System from Promega utilizes differing luminescent properties of firefly and *Renilla* luciferase to normalize activity of an experimental reporter with an internal control. The FlexStation 3 reader, with liquid transfer capabilities, provides a robust platform for increased throughput of this important flash luminescence assay. It expands capacity to perform real time fast kinetic assays by pipetting and reading simultaneously.

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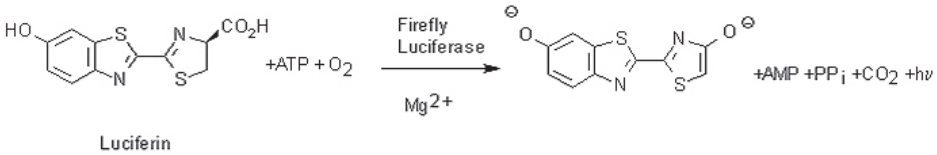
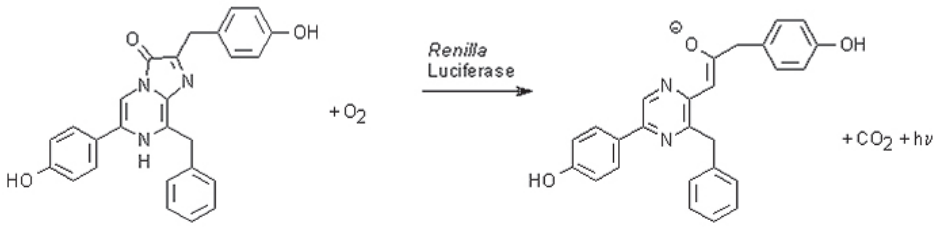
- Easily changed 8- and 16-channel pipette heads enable assays in both 96- and 384-well plates
- Multiple additions can be made to the same well enabling agonist and antagonist assays
- Assays are easily optimized with user defined addition and trituration parameters
- Less dead volume saves precious reagents compared to injectors
- Triturating in the well after addition facilitates mixing in low volume assays



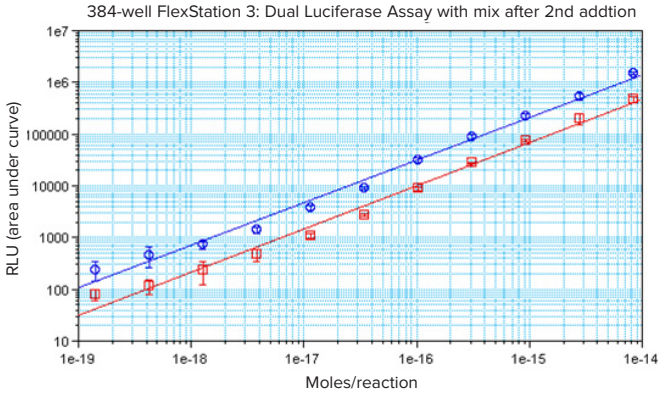
Luciferin



Coelenterazine

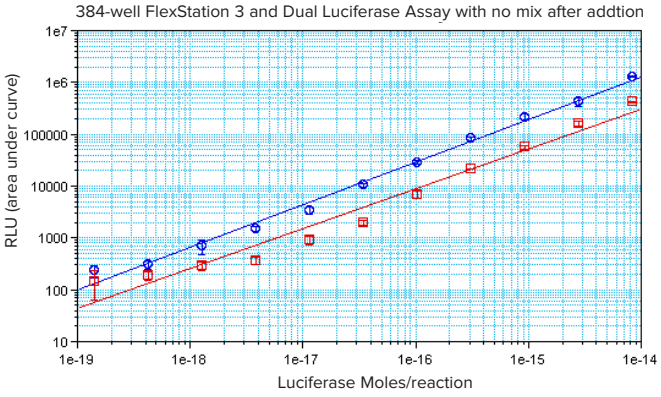
Dual-luciferase assay principle. The firefly and *renilla* luciferase reactions are shown above. The firefly enzyme catalyzes the ATP-, Mg^{2+} and O_2-dependent oxidation of luciferin with the concomitant release of light. *Renilla* luciferase catalyzes the O_2-dependent oxidation of coelenterate luciferin (coelenterazine) but does not require Mg^{2+} or ATP.

Trituration after addition



In 384-well mode, a trituration step was added after pipetting each reagent increasing linearity at lower concentrations of luciferase. Firefly luciferase LLD= 0.72 attomoles, R^2= 0.99 and *Renilla* luciferase LLD= 0.57 attomoles, R^2= 0.989 .

No trituration after addition



In 384-well mode, no trituration step was added after pipetting each reagent. Firefly luciferase LLD= 0.69 attomoles, R^2= 0.993, and *Renilla* luciferase LLD= 40 attomoles, R^2= 0.966. Linearity of *Renilla* luciferase was affected at lower concentrations due to lack of mixing.

Comparison of Photina luminescent calcium mobilization assays

Calcium-activated photoproteins are important tools for detecting receptor-mediated signaling events involving calcium mobilization in mammalian cells. One major advantage of photoproteins is the immediate emission of flash luminescence upon calcium binding to the coelenterazine-photoprotein complex. The background signal of Photina measurements is close to zero, resulting in high signal-to-background ratios. Furthermore, the luminescent light emitted by the oxidation of coelenterazine does not depend on optical excitation, eliminating issues with auto-fluorescence.

This study provides a basic protocol for performing a calcium flux assay with Photina and for determining the concentration response of IMETIT in adherent CHO mito-Photina/H3 cells at various cell concentrations using the FlexStation 3 reader.

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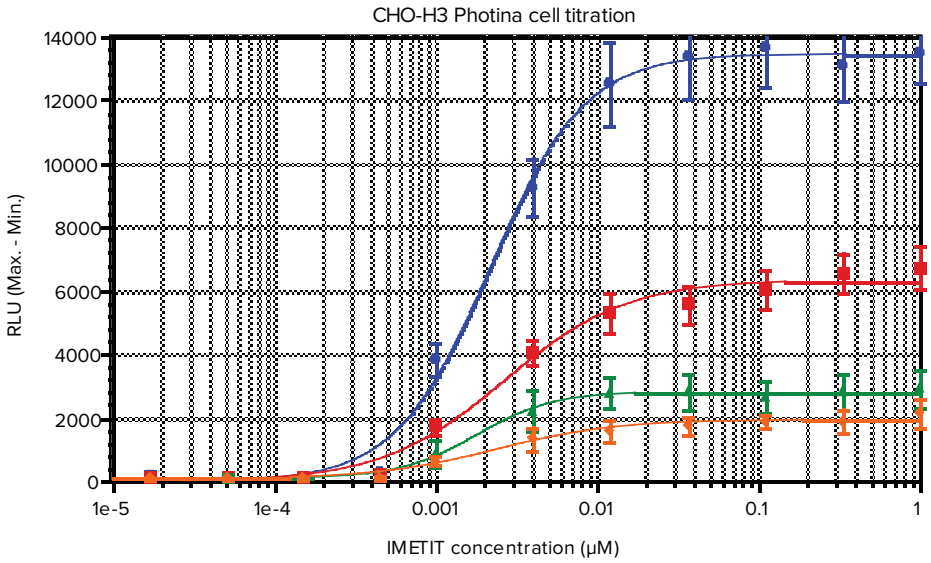
- Flexible solution for early identification of lead compounds in the drug discovery process
- Permits real-time measurement of fluorescent & luminescent cell-based assays one column at a time in 96- or 384-well formats
- Preconfigured aequorin assay protocol is available with SoftMax Pro Software



CHO-H3 Photina cell titration. CHO mito-Photina/H3 cells were plated at varying cell concentrations (5000 (•), 2500 (•), 1250 (•), and 625 (•) cells/well) in 384- well, black-wall, clear-bottom plates. The FlexStation 3 System added agonist during real-time luminescent detection. Results are the average of approximately 16 replicates.

Measurement of calcium signaling

FLIPR® Calcium Assay Kits employ sensitive calcium indicators and proprietary masking dyes to enable you to conduct highly sensitive fluorescent screens of G-protein coupled receptors (GPCRs), ion channels, and other calcium sensitive targets. By using a novel dye formulation to further enhance the calcium flux assay signal window, assay robustness is increased, while providing greater assay protocol flexibility. As a result, the FLIPR Calcium 6 and Calcium 6-QF Calcium Kit dyes become more suitable for measuring calcium flux in 384-well plates with the medium throughput FlexStation 3 reader using the 16 channel pipettor.

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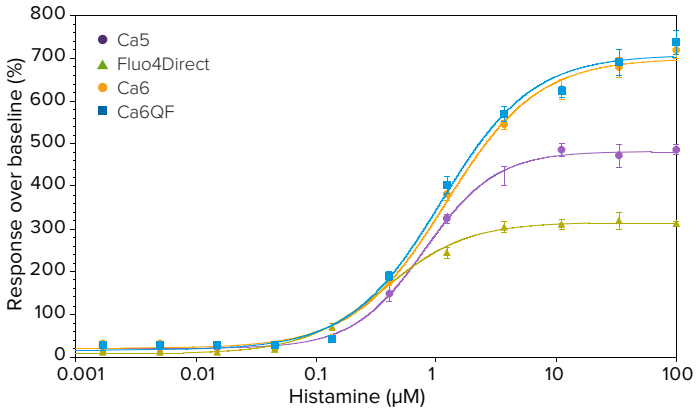
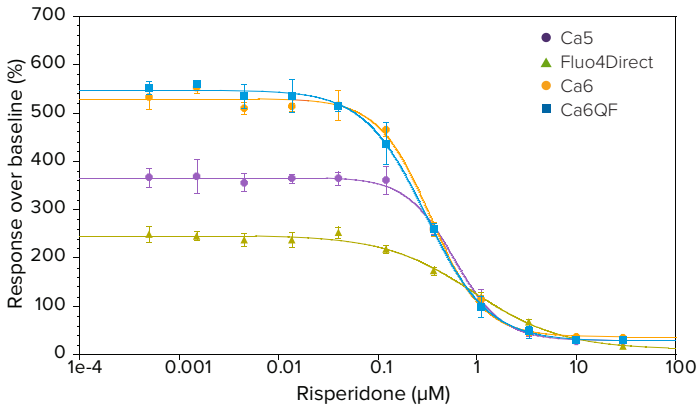
Assay Kits Compatibility Table

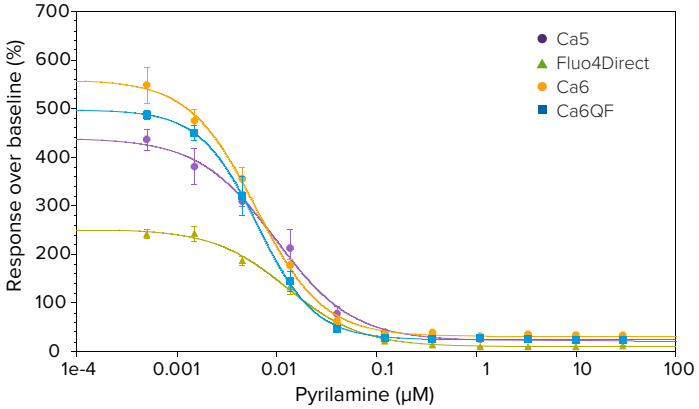
- Provides largest signal window of comparable calcium kits and dyes
- Enables low signal screens, including endogenous, primary or stem cell targets
- Masking technology significantly reduces extracellular background with proprietary one-step protocol
- Resistance to organic ion transporters minimizes need for anion reuptake inhibitors



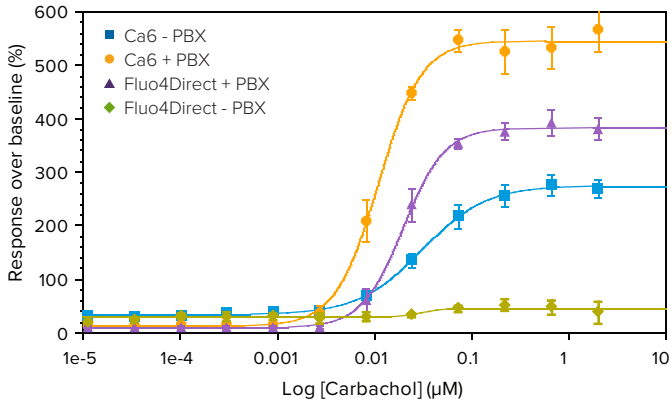
FLIPR Calcium 6 and 6-QF Assay Kits provide the largest signal window. Histamine H1 is an endogenous receptor in HeLa cells. Comparing FLIPR Calcium 6 and 6-QF to other dyes shows that both had the largest signal window. EC_{80} values were within one-half log and Z factors at EC_{80} were comparable.



Risperidone antagonist response to histamine challenge in HeLa cells. Risperidone is an antipsychotic drug used to treat schizophrenia. It is a dopamine antagonist that also has antihistamine properties. The signal from both Calcium 6 and 6-QF dyes provides the largest window for the antagonist assay. IC_{50} values are within one-half log of each other and Calcium 6-QF has the highest Z factor at IC_{50} concentration.



Pyrilamine antagonist response to histamine challenge in HeLa cells. Pyrilamine is a first generation Histamine H1 antagonist. Because of the larger signal window provided by the FLIPR Calcium 6 Assay Kit, the Z factor at IC_{50} is the largest. In addition, the Calcium 6-QF kit also provides a robust assay that does not require washing when quench sensitive targets are to be studied.



FLIPR Calcium 6 assay dye does not require use of anion reuptake inhibitors. Assay performed with CHO-M1 cells and Calcium 6 dye demonstrates a response to carbachol without the need for incubation with probenecid. The corresponding assay with Fluo-4 Direct shows virtually no signal. Calcium 6 dye is an important new development for understanding targets that may be sensitive to anion reuptake inhibitors.

Optimization of a muscarinic M_3-receptor assay using frozen CHO cells

Cell-based assays can often be challenging and time consuming. To facilitate and streamline this complicated process, frozen cells, which can be assayed without prior cultivation, have become a suitable and frequently used alternative to cells in continuously growing culture. The FlexStation 3 reader in combination with the FLIPR Calcium 5 Assay Kit optimally measure changes in intracellular Ca^{2+} in frozen CHO cells expressing the muscarinic M_3 receptor. The dual monochromators facilitate optimal excitation and emission wavelength selection, and allow both single-wavelength and dual-wavelength ratiometric indicators to be used.

- Versatile assay offers broad applicability across a range of biological targets
- Homogeneous format reduces plate handling and provides higher throughput
- Multi-channel liquid handling enables easy setup of both agonist and antagonist studies

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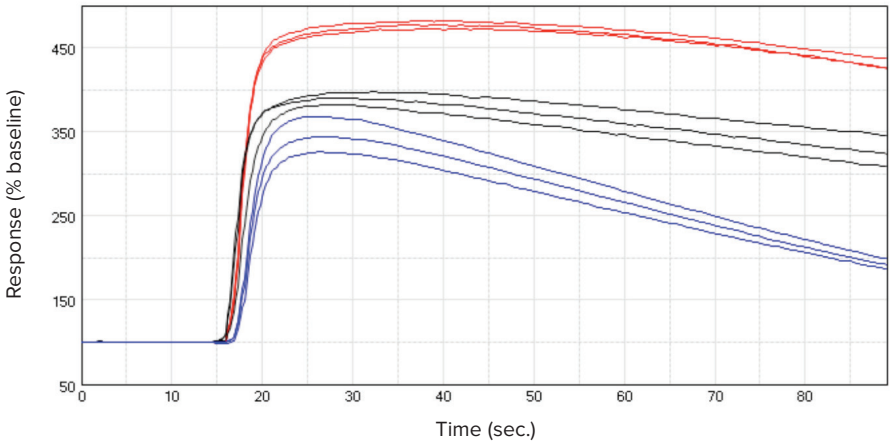
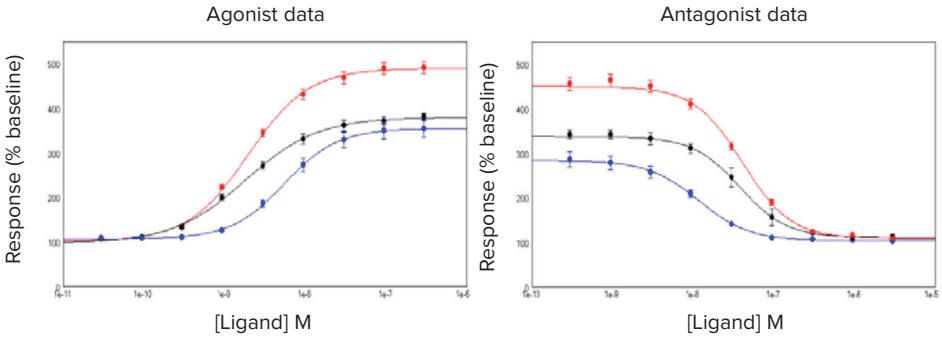
Assay Kits Compatibility Table



Kinetic traces. Representative kinetic traces exported from SoftMax Pro 6 Software for ACh (300 nM) in FLIPR Calcium 5 Assay Kit loaded CHRM3 cells on the FlexStation 3 reader. Cell conditions shown were (•) cells in continual culture, (•) frozen cells used 18 hours after thawing or (•) cells used from frozen. Plot shows % response over baseline on the y-axis against time in sec. on the x-axis.



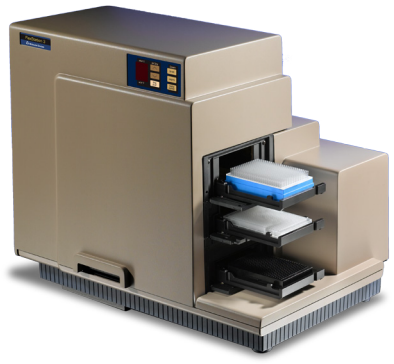
Activation/inhibition curves. Activation/Inhibition curves for ACh ± *p*-F-HHSiD in FLIPR Calcium 5 Assay Kit loaded CHRM3 cells. Cell conditions tested were (•) cells in continual culture, (•) frozen cells used 18 hours after thawing or (•) cells used from frozen.

FlexStation 3 Multi-Mode Microplate Reader configurations

We offer a range of consumables and assay kits to enable application flexibility you require to support your research.

Ordering information	Description	Part number
FlexStation 3 Reader	• FlexStation 3 Base System • SoftMax Pro Software • 1-year warranty	FLEX3
Pipettor head kit, 8-channel (96) for FlexStation 3 Reader	• 8-channel pipettor • (10) racks of 96-well, FlexStation Pipet Tips (Black) • 96-well yellow plate	0200-6182
Pipettor head kit, 16-channel (384) for FlexStation 3 Reader	• 16-channel pipettor • (10) racks of 384-well, FLIPR® Tetra Pipet Tips (Clear) • 384-well yellow plate	0200-6183
Consumables		
96-Well, FlexStation Pipet Tips (Black)	• 200 µL capacity • (10) racks/box	9000-0911
96-Well, FlexStation Pipet Tips (Clear)	• 200 µL capacity • (10) racks/box	9000-0912
384-Well, FLIPR Tetra Pipet Tips (Black)*	• 30 µL capacity • (50) racks/case	9000-0764
384-Well, FLIPR Tetra Pipet Tips (Clear)*	• 30 µL capacity • (50) racks/case	9000-0763

* Inquire regarding partial case purchases.



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