



Calcium Assay Kits

Superior detection for a diversity of targets

Key Features

- Optimized no-wash assays
- Compatible with endogenous, primary or stem cell targets
- Rapid assay development
- Patented quench technology
- Largest signal window available
- Quench- or probenecid-free protocols

Since introduction in 1999, Molecular Devices® Calcium Assay Kits are the platform of choice for measuring changes in intracellular calcium during drug discovery and research. Built as the most comprehensive calcium portfolio, the FLIPR® and QBT™ Calcium Assay Kits deliver pre-optimized, homogeneous, fluorescence-based formulations to expedite assay development and screening of GPCR and ion channel targets.

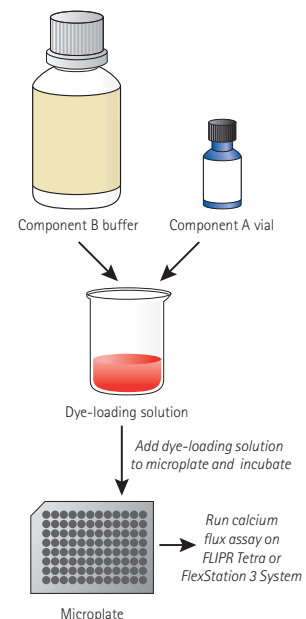
Optimized Homogeneous Assays

This generous portfolio offers seven formulations to surmount even your most difficult assays, including frozen, primary or stem cells; transient, endogenous or low-expression receptors; plus chemokines, allosteric modulators, or partial agonists. Formulations match a unique fluorophore with our proprietary masking technology, providing optimal conditions for your target of interest.

Rapid Assay Development

Utilizing a mix-and-read procedure, cells are incubated with the assay kit, and then transferred directly to the instrument for evaluation. No intermediate wash steps are required; thereby

eliminating cell detachment, lowered cell competency and incomplete dye removal that may contribute to data variability. Protocols are pre-validated for use on FLIPR® and FlexStation® Systems to conserve your time while quickly advancing future discoveries.



Proprietary masking technology

Higher throughput capacity

Molecular Devices Calcium Assay Kits boost the throughput of cell-based screens by 33% over standard wash protocols by eliminating cumbersome wash steps and related preparation time required for wash buffers and calibrations (when running 20+ plates). This combined with the exclusion of potential dispensing and washing errors or associated equipment failures further insures the integrity of screening operations.

These kits are compatible with room temperature (FLIPR Calcium 4 and 5 Kits) and long-duration (several hours) incubations to further extend the assay's unattended throughput, making it conducive to batch loading during automated high throughput screens.

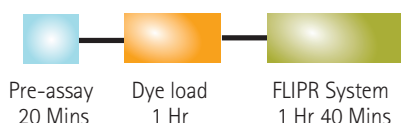
Patented technology

Calcium Assay Kits from Molecular Devices employ a quenching dye to reduce background fluorescence and improve the signal-to-noise ratio. The patented quench technology (patent no. US 6,420,183) is offered to drug discovery and life science researchers by Molecular Devices, through the purchase of FLIPR and QBT Calcium Assay Kits.

Conventional calcium assay



FLIPR or QBT Calcium Assay Kit



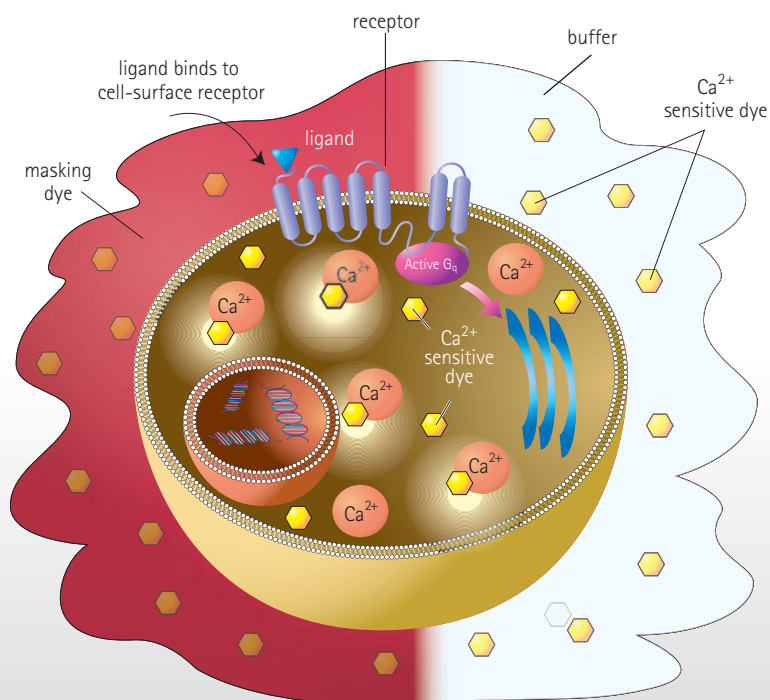
Molecular Devices' Calcium Assay Kits reduce assay time for 20 plates from 4 hours 55 minutes to 3 hours.

Standard

Significantly reduce fluorescence background with one-step protocol

Quench-Free

For quench-sensitive targets and multiplexing

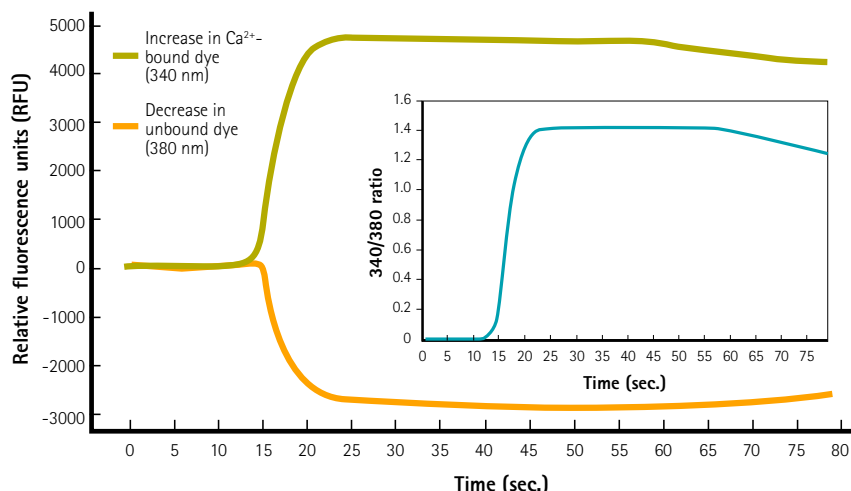


Increase in cytosolic Ca²⁺ can be detected by FLIPR or FlexStation Systems using calcium-sensitive dye indicators

Each calcium assay kit utilizes a unique calcium sensitive dye that is absorbed into the cell's cytoplasm during incubation. Upon target activation, intracellular calcium is released, binding with the dye, thereby increasing fluorescence intensity. Molecular Devices' calcium kits utilize an extracellular masking technology to block background fluorescence and increase your assay's signal window. FLIPR Calcium 6-QF Assay Kit is the one exception, enabling assay performance without a masking technology for quench-sensitive applications.

Fura-2 QBT Calcium Kits

Fura-2 QBT Calcium Kit: ratiometric analysis

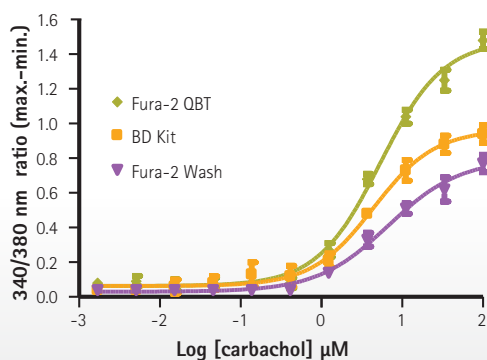


Fura-2 QBT Dye is excited at 340 nm and 380 nm, with emission at 510 nm. Upon calcium flux, the cytoplasm's calcium concentration increases, causing the fluorescence intensity of calcium-bound Fura-2 at 340/510 nm to increase proportionately. In contrast, the 380/510 nm intensity declines in synchronization with the diminishing concentration of unbound dye. By ratioing signal traces from the two wavelengths, artifacts associated with uneven dye distribution and cell number variation are minimized because they effect both measurements equally.

For researchers concerned about fluorescent artifacts, the Fura-2 QBT Calcium Kit is a simple, mix-and-read format that employs ratiometric excitation (340/380 nm) to accurately measure calcium mobilization (510 nm). Leveraging our proprietary masking technology with the industry-standard Fura-2 ratiometric calcium indicator, researchers can:

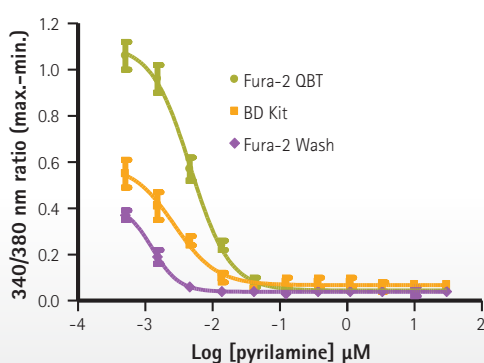
- See largest Fura-2 signal window available
- Eliminate wash artifacts and increase throughput with homogeneous assay
- Minimize the impact of uneven dye loading and leakage on results
- Interrogate low-density, weakly, or non-adherent cells using no-wash protocol
- Validated for use on FlexStation® 3 and FLIPR® Tetra Systems
- Reference thousands of citations where Fura-2 has been used for cell lines and targets

Agonism of the muscarinic M3 receptor in HEK-293 cells



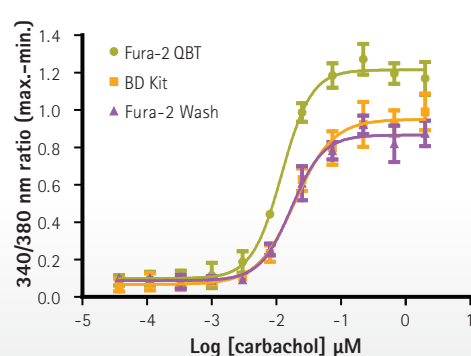
Carbachol	Fura-2 QBT	BD Kit	Fura-2 Wash
EC ₅₀ (μM)	5.3	4.4	6.8
Z @ EC ₈₀	0.77	0.74	0.65
Window	1.4	0.9	0.76

Antagonism of the histamine H1 receptor in HeLa cells



Pyrilamine	Fura-2 QBT	BD Kit	Fura-2 Wash
EC ₅₀ (nM)	4.7	2.7	1.3
Z @ EC ₈₀	0.8	0.49	0.33
Window	1.1	0.52	0.37

Agonism of the muscarinic M1 receptor in CHO-M1 cells



Carbachol	Fura-2 QBT	BD Kit	Fura-2 Wash
EC ₅₀ (nM)	13	17	19
Z @ EC ₈₀	0.7	0.5	0.38
Window	1.1	0.85	0.7

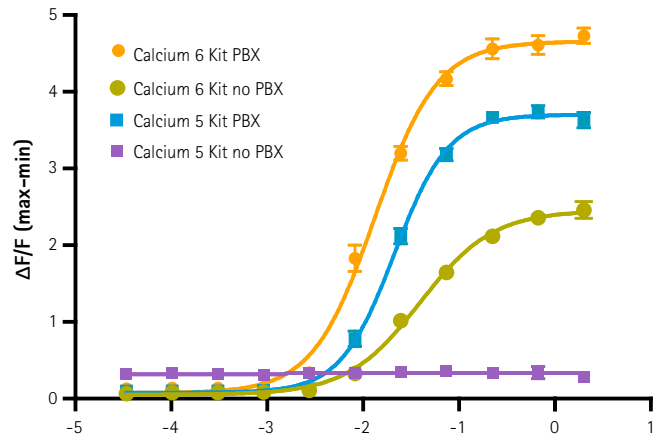
The Fura-2 QBT Calcium Kit provides the largest 340/380 nm ratio signal window and Z factor at EC₈₀ when compared to BD Ratiometric Calcium Kit and the traditional Fura-2 wash assay.

FLIPR Calcium 6 Assay Kits

Utilizing a novel fluorescent indicator dye technology, the FLIPR Calcium 6 and 6-QF Assay Kits provide the largest signal window on the market, enabling researchers to:

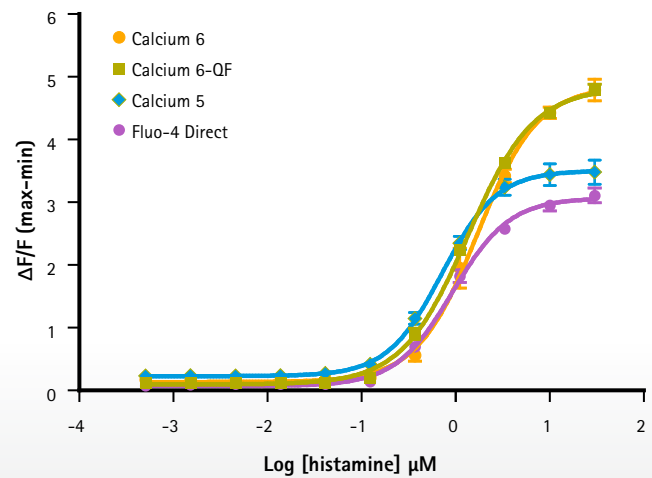
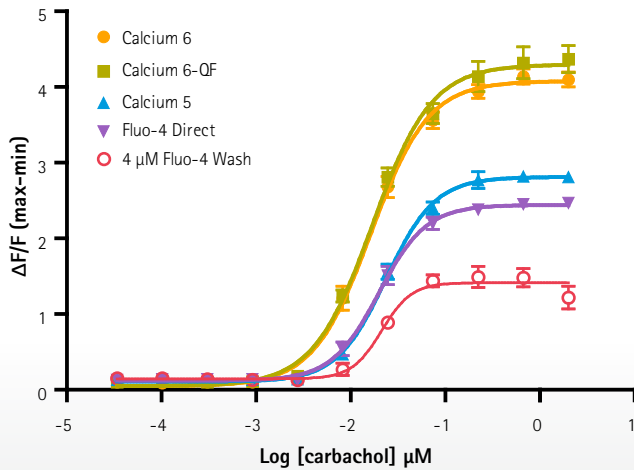
- Rekindle low signal screens; including endogenous, primary or stem cell targets
- Improve assay quality, with or without masking dye technology
- Multiplex assays using quench-free option to better characterize signal pathways
- Understand target behavior in the presence of an organic anion transporter using probenecid optional protocol

Anion exchange protein resistance



Novel fluorophore is more resistant to organic anion exchange protein, such as probenecid (PBX), enabling FLIPR Calcium 6 Assay Kit to produce a stronger signal in the absence of probenecid, while conserving EC_{50} values and Z-factors at $EC_{80} > 0.5$. CHO-M1 cells pictured.

FLIPR Calcium 6 Assay Kit: highest signal-to-noise ratio



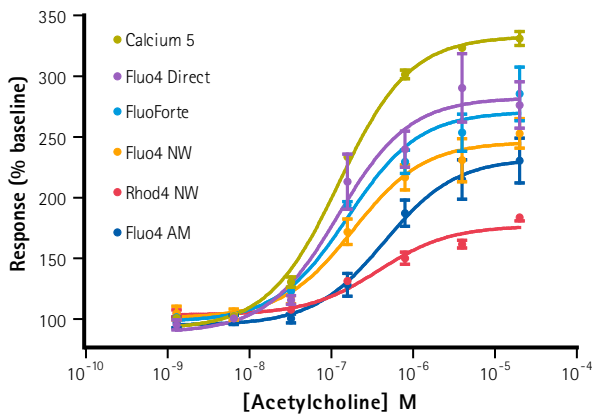
Comparison of FLIPR Calcium 6 Assay Kits to other calcium indicators was measured using agonism of the muscarinic receptor on CHO M1 cells (left) and agonism of endogenous histamine H1 receptor on HeLa cells (right).

Other FLIPR Calcium Assay Kits

FLIPR Calcium 5 Assay Kit

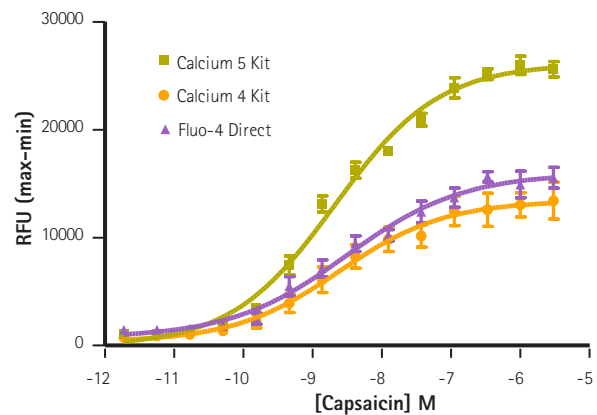
The FLIPR Calcium 5 Assay Kit utilizes a unique fluorophore that exhibits a large signal window, and is amenable to incubation at 37°C or room temperature, facilitating fully-automated screens that may run overnight.

FLIPR Calcium Assay Kit signal window comparison in 1321N1 cells



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.

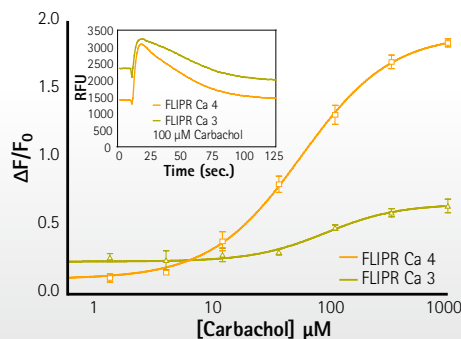
FLIPR Calcium 5 Assay Kit signal window comparison in hTRPV1-HEK-293



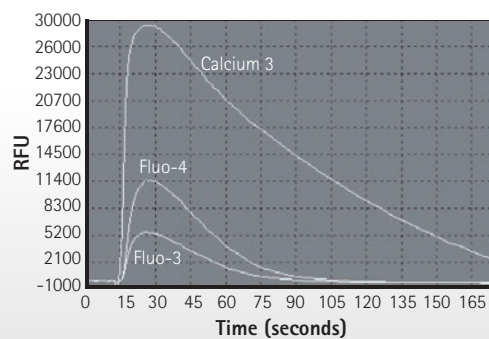
ChanTest hTRPV1-HEK-293 cells are a stably transfected cell line with a human TRPV1 transient receptor potential channel constitutively expressed in the HEK-293 cell line. Cells were incubated with dye for one hour, then ligand was added during detection on the FLIPR Tetra System to cause an influx of calcium and an increase in fluorescence.

FLIPR Calcium 4, 3 and 1 Assay Kits

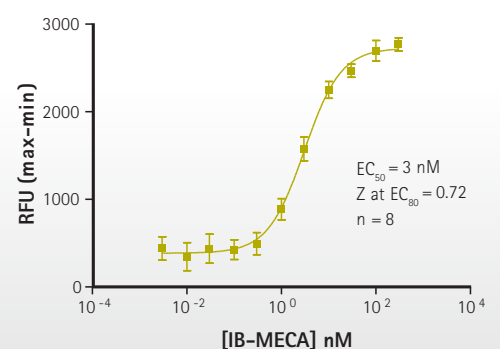
FLIPR Calcium 4 Assay Kit novel masking dye enhances signal-to-noise ratio (Figure A)



FLIPR Calcium 3 Assay Kit demonstrates superior results from chemokine and small peptide targets (Figure B)



FLIPR Calcium Assay Kit to monitor IB-MECA agonism in CHO-K1 adenosine A3 cells (Figure C)



A: Reduced background in the FLIPR Calcium 4 Assay Kit (orange) leads to improved $\Delta F/F_0$ values in an assay with especially small calcium response (endogenous response to carbachol in HEK 293 cells). **B:** Comparison of signal intensity obtained with FLIPR Calcium 3 Assay Kit vs. Fluo-4 and Fluo-3 wash protocols. Response of CHO cells transfected with M1-muscarinic receptors to 100 nM carbachol. **C:** Response to Adenosine A3 receptor agonist, IB-MECA, in CHO K1 cells transfected with Adenosine A3 receptor and promiscuous G_α₁₆ to force signalling through Ca²⁺ flux. Figures A and C assays were run on the FLIPR Tetra System, while Figure B assay was run on the FLIPR 3 System.

Selecting the ideal calcium kit

FLIPR and QBT Calcium Assay Kits are available in several formulations and sizes to support your unique cell line, target or throughput requirement. Most assay kits are compatible with 96-, 384-, and 1536-well plate configurations.

	Evaluation Kit	Explorer Kit	Bulk Kit	Express Kit*
Ideal use	Selection of optimal formulation for cell line or target	Assay development or small-scale experiments	Sufficient for medium to large screens	Preferred for fully automated screens that process > 50 plates per run
Component A	3 vials per formulation	10 vials	10 vials	2 vials
# Plates	varies	10	100	100

Note: Detailed product configuration available at www.moleculardevices.com/calcium

* Express kit configuration available for FLIPR Calcium 3, 4, 5, 6 and 6-QF Assay Kits.

Ordering information

Product description	Item code			
	Explorer	Bulk	Express	Evaluation
Fura-2 QBT Calcium Kit	R8197	R8198		R6139
FLIPR Calcium 6 Assay Kit	R8190	R8191	R8195	
FLIPR Calcium 6-QF Assay Kit	R8192	R8193	R8196	
FLIPR Calcium 6 and 6-QF Assay Evaluation Kit				R8194
FLIPR Calcium 5 Assay Kit	R8185	R8186	R8187	
FLIPR Calcium 4 Assay Kit	R8142	R8141	R8143	
FLIPR Calcium 3 Assay Kit	R8091	R8090	R8108	
FLIPR Calcium 3, 4, and 5 Assay Evaluation Kit				R8172
FLIPR Calcium Assay Kit	R8041	R8033		

Contact Us

Phone: +1-800-635-5577
 Web: www.moleculardevices.com
 Email: info@moldev.com

Check our website for a current listing of worldwide distributors.

Regional Offices

USA and Canada +1-800-635-5577
 Brazil +55-11-3616-6607
 China (Beijing) +86-10-6410-8669
 China (Shanghai) +86-21-3372-1088
 Germany 00800-665-32860

Japan (Osaka) +81-6-7174-8831
 Japan (Tokyo) +81-3-6362-5260
 South Korea +82-2-3471-9531
 United Kingdom +44-118-944-8000

