APPLICATION NOTE

Fura-2 QBT Calcium Kit: A homogenous Fura-2 calcium assay

Introduction

Fura-2 dye has long been considered an important tool to measure calcium mobilization in cellular imaging, GPCR-mediated intracellular calcium flux, and ion channel activation. This ratiometric dye helps correct for assay inconsistencies in dye loading or cell plating through calculating the fluorescence intensity ratio between bound and free indicators. However washing is required, increasing well-to-well variability and adding time and complexity to each assay.

The Fura-2 QBT™ Calcium Kit from Molecular Devices incorporates proven quench based technology with a ratiometric Fura-2 calcium indicator to provide a homogenous assay to minimize cell based variability, while increasing throughput through elimination of cell washing prior to detection. In addition, the Fura-2 QBT Calcium Kit excites in the ultraviolet spectrum, enabling researchers to mitigate fluorescent compounds that are excited at 488 nm.

Assay principle

Fura-2 AM is a calcium sensitive indicator that is excited at 340 nm and 380 nm, with emission at 510 nm. The dye undergoes an absorption shift from 380 nm to 340 nm when bound to calcium ions. Figure 1 shows an increase in calcium bound to Fura-2 causes the 340/510 nm emission signal (green) to increase while the 380/510 nm emission signal (orange) decreases. The ratio of the emission signals (inset plot) is calculated to measure the maximum-minimum signal response for the concentration response curves.

Fura-2 QBT Calcium Kit assay preparation

Fura-2 QBT Calcium Kit, Explorer format (PN #R8197) was used in this study. Dye was resuspended in Hank's Balanced Salt Solution (HBSS) with 20 mM HEPES. Probenecid (PBX) was added where necessary to inhibit dye leaking out of cells. Adherent CHO M1 or HeLa cells plated the night before, in 384-well black and clear cell plates, were removed from the incubator. 25 µL Fura-2 QBT dye or competitor kit dye loading buffer was added to wells containing 25 µL assay buffer or media. Plates loaded with the Fura-2 QBT Kit (PN #R8197) or BD Ratiometric Calcium Kit (#644243) were incubated one hour at 37°C, 5% CO_2. A traditional Fura-2 wash protocol method was also used for comparison studies.

Benefits

- **Larger signal window is provided by quench-based technology**
- **Assay variability is decreased with removal of wash steps and ratiometric measurement**
- **Save time and assay costs by removing wash steps**

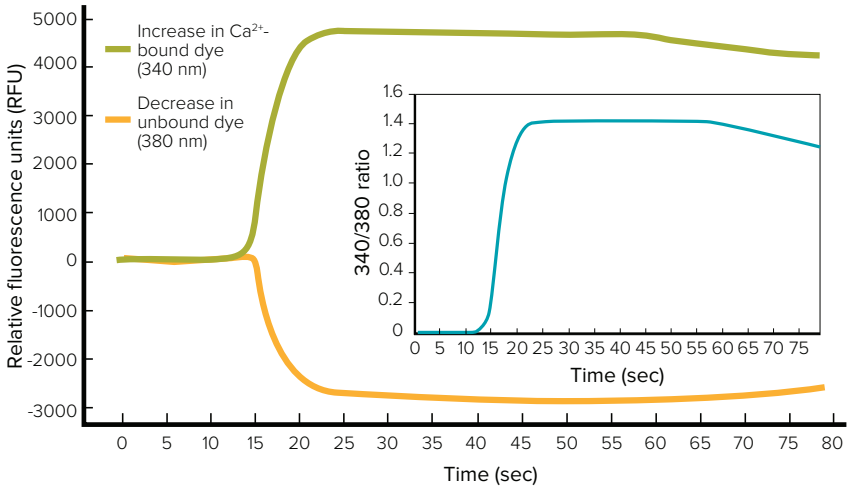


Figure 1. Ratiometric analysis with FURA-2 QBT Calcium Assay. Raw signal changes, in response to changes in the intracellular calcium concentrations, are shown in orange and green. The ratio between the two is shown in the inset.

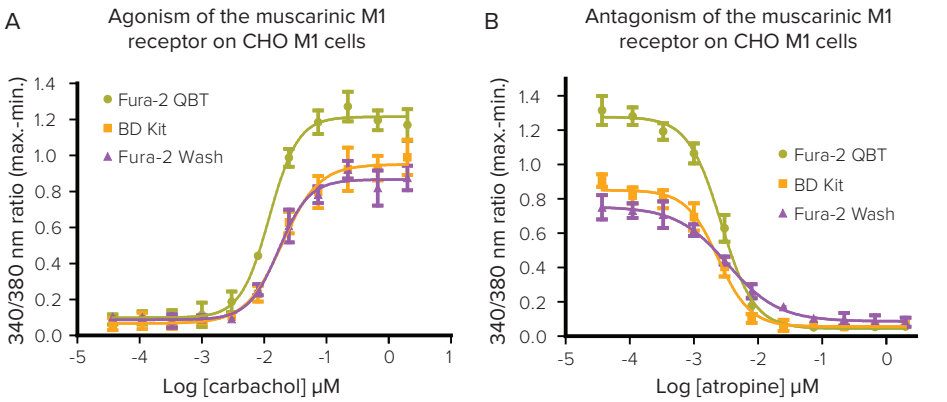
When the traditional Fura-2 wash method was used, media was removed from each well prior to adding 50 µL 1X dye loading buffer then cell plates were incubated using the same time and conditions as other kits. All plates were removed from the incubator and allowed to cool to room temperature 10 minutes prior to being read on either the FlexStation® 3 Multi-Mode Microplate Reader or FLIPR® Tetra System. Plates using traditional Fura-2 wash protocol method required washing 3X with HBSS assay buffer to remove excess dye before being read.

Ratiometric calcium mobilization assay on FlexStation 3 reader and FLIPR Tetra System

A 5X concentration of appropriate ligand was prepared in HBSS buffer + 20 mM HEPES in 384-well polypropylene plates. Agonist was added during detection on the FlexStation 3 reader (Figure 2) or FLIPR Tetra System (Figure 3) using parameters optimized for a ratiometric assay. Dye excitation was carried out at 340 and 380 nm wavelengths.

Emission signals at 510 nm were detected, then measured in Relative Fluorescence Units (RFU) for both excitation wavelengths in each well for approximately 90 seconds, including during and post addition. Output calculated was the ratio between 340 nm and 380 nm wavelength signals at each time point during the assay. From the ratiometric signal trace, maximum - minimum value was calculated. Data for graphs and EC_{50}/IC_{50} concentrations was exported from ScreenWorks® or SoftMax® Pro Software, then calculated using GraphPad Prism. Z factor calculations were performed using the method described by Zhang, et. al.

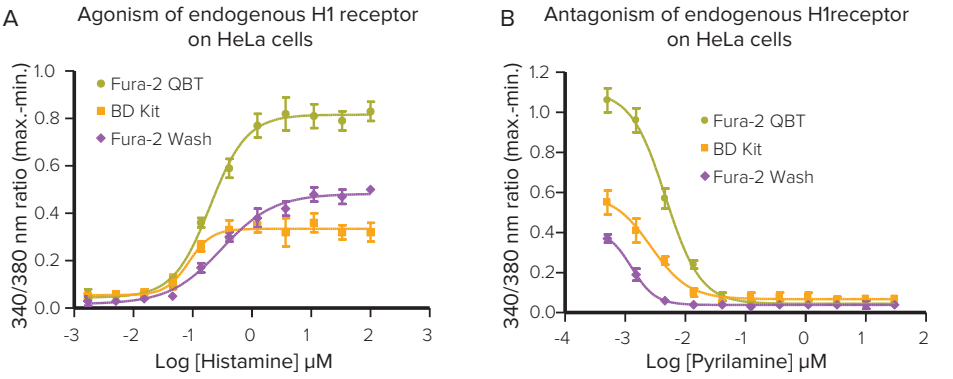
Results



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

Atropine	Fura-2 QBT	BD Kit	Fura-2 Wash
IC_{50} (nM)	2.4	2.2	3.3
Z @ IC_{80}	0.81	0.53	0.66
Window	1.2	0.75	0.72

Figure 2. Fura-2 QBT Calcium Kit provides the largest signal window. The kit provides the largest signal window and most robust Z factors at EC_{80} or IC_{80} on the FlexStation 3 reader.



Histamine	Fura-2 QBT	BD Kit	Fura-2 Wash
EC_{50} (nM)	200	900	300
Z @ EC_{80}	0.72	0.54	0.69
Window	0.77	0.29	0.44

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

Figure 3. Fura-2 QBT Calcium Kit assay helps remove assay variability. The kit further enhances the assay by providing a larger signal window, robust Z factors at EC_{80} or IC_{80}, and removing assay variability by removing wash steps. The assay was measured using the FLIPR Tetra System with UV LEDs.

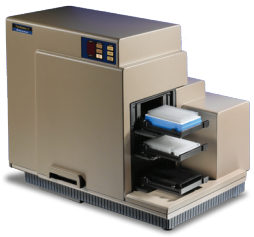
Conclusion

Fura-2 QBT Calcium Kit offers a homogenous alternative to the traditional Fura-2 assay by eliminating the need for washing through use of the Molecular Devices proprietary quench-based technology. This improves throughput, reduces assay variability associated with uneven cell plating or loss of cells during washing, and eliminates assay costs (buffers and requirement for assay repeats due to variability). Compared to BD Ratiometric Calcium Kit and traditional Fura-2 wash assay, the Fura-2 QBT Calcium Kit provides the largest signal window and strongest Z factors at EC_{80} and IC_{80}. Because the Fura-2 dye excites in the UV range, the kit avoids interference from compounds that auto-fluoresce when excited at 488 nm. The Fura-2 QBT Calcium Kit is validated for use with the FlexStation 3 reader and FLIPR Tetra System, providing a scalable solution from a bench top assay to full high throughput screen.

Reagent	Description	Part number
Fura-2 QBT Calcium Sample Kit	(2) vials of component A* (1) bottle of dilution buffer (Component B) * Each reagent vial (Component A) is sufficient for 1 plate (96-, 384-, 1536-well). Each kit is sufficient for 2 plates.	R6139
Fura-2 QBT Calcium Explorer Kit	(10) vials of component A* (1) bottle of dilution buffer (Component B) * Each reagent vial (Component A) is sufficient for 1 plate (96-, 384-, 1536-well). Each kit is sufficient for 10 plates.	R8197
Fura-2 QBT Calcium Bulk Kit	(10) vials of component A* * Each reagent vial (Component A) is sufficient for 10 plates (96-, 384-, 1536-well). Each kit is sufficient for 100 plates.	R8198

Compatible with these Molecular Devices systems

FLIPR® Tetra System



FlexStation® 3 Multi-Mode Microplate Reader

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