Screen Quest™ Fura-2 No Wash Calcium Assay Kit

Catalog number: 36320, 36321
Unit size: 10 Plates, 100 Plates

Component	Storage	Amount (Cat No. 36320)	Amount (Cat No. 36321)
Component A: Fura-2 AM	Freeze (< -15 °C), Minimize light exposure	1 vial, lyophilized	10 vials, lyophilized
Component B: 10X Pluronic F127 Plus	Freeze (< -15 °C), Minimize light exposure	10 bottles (1 mL/bottle)	10 bottles (10 mL/bottle)
Component C: HHBS (Hanks' buffer with 20 mM Hepes)	Freeze (< -15 °C), Minimize light exposure	1 bottle (100 mL)	Not included

OVERVIEW

Calcium flux assays are preferred methods in drug discovery for screening G protein coupled receptors (GPCR). This ratiometric calcium assay kit allows homogeneous measurement of intracellular calcium changes caused by activation ofG-protein-coupled receptors or calcium channels. Cells expressing a GPCR of interest that signals through calcium are pre-loaded with Fura-2 AM which can cross cell membrane. Once inside the cell, the lipophilic blocking groups of Fura-2 AM are cleaved by esterases, resulting in a negatively charged fluorescent dye that stays inside cells and its fluorescence wavelength is blue-shifted upon binding to calcium. When cells stimulated with agonists, the receptor signals the release of intracellular calcium, which greatly increase the fluorescence intensity of Fura-2 at the short wavelength. The ratiometric characteristics of Fura-2 make this kit an ideal tool for more accurate measurement of cellular calcium concentration compared to Fluo-4 of the single wavelength. With a single addition, the assay is easy to perform and desirable in a high thoroughput environment. The assay can be used in a convenient 96-well or 384-well microtiter-plate format and readily adapted to automation.

AT A GLANCE

Protocol Summary

1. Prepare cells in growth medium
2. Add Fura-2 AM dye-loading solution (100 µL/well for 96-well plate or 25 µL/well for 384-well plate)
3. Incubate at room temperature for 1-2 hour
4. Monitor fluorescence intensity at Ex/Em = 340/510 nm and 380/510 nm

Important Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Fluorescence microplate reader

Excitation	340/380 nm
Emission	510 nm
Cutoff	470 nm
Recommended plate	Black wall/Clear bottom
Instrument specification(s)	Bottom read mode/Programmable liquid handling

Other instruments

FDSS, FLIPR, FlexStation

CELL PREPARATION

For guidelines on cell sample preparation, please visit https://www.aatbio.com/resources/guides/cell-sample-preparation.html

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. Fura-2 AM stock solution
Add 200 µL of DMSO into the vial of Fura-2 AM (Component A), and mix them well.

Note 20 µL of Fura-2 AM stock solution is enough for one plate. Unused Fura-2 AM stock solution can be aliquoted and stored at -20 ° C for more than one month if the tubes are sealed tightly. Protect from light and avoid repeated freeze-thaw cycles.

2. Assay Buffer (1X)
a) For **Cat. # 36320 (10 plates kit)** , make 1X assay buffer by adding 9 mL of HHBS (Component C) into 10X Pluronic® F127 Plus (1 mL, Component B), and mix them well.b) For **Cat. # 36321 (100 plates kit)** , make 1X assay buffer by adding 90 mL of HHBS (Not included) into 10X Pluronic® F127 Plus (10 mL, Component B), and mix them well.

Note 10 mL of 1X assay buffer is enough for one plate. Aliquot and store un-used 1X assay buffer at -20 ° C. Protect from light and avoid repeated freeze-thaw cycles.

PREPARATION OF WORKING SOLUTION

Fura-2 AM dye-loading solution
Add 20 µL of Fura-2 AM stock solution into 10 mL of 1X assay buffer, and mix them well.

Note This working solution is stable for at least 2 hours at room temperature.

SAMPLE EXPERIMENTAL PROTOCOL

1. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of Fura-2 AM dye-loading solution into the cell plate. *Note:* If your compounds interfere with the serum, then it is important to replace the growth medium with HHBS buffer.

2. Incubate the dye-loading plate in a cell incubator for 1 hour, and then incubate the plate at room temperature for another 20 minutes. *Note:* If the assay requires 37 ° C, perform the experiment immediately without further room temperature incubation.

3. Prepare the compound plate with HHBS or your desired buffer.

4. Run the calcium flux assay by monitoring the fluorescence increase at Ex/Em = 340/510 nm and 380/510 nm. *Note:* It is important to run the signal test before the experiment. Different instruments have their own intensity range. *Note:* For assays performed on FDSS, use the standard filters for Fura-2 calcium assays on the instrument.

EXAMPLE DATA ANALYSIS AND FIGURES

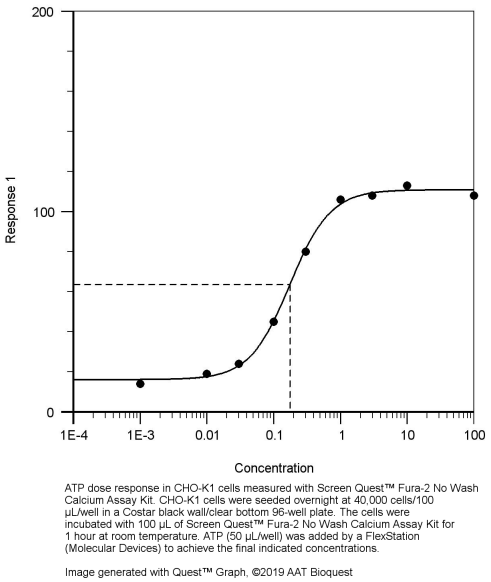

Figure 1. ATP dose response in CHO-K1 cells measured with Screen Quest™ Fura-2 No Wash Calcium Assay Kit. CHO-K1 cells were seeded overnight at 40,000 cells/100 µL/well in a Costar black wall/clear bottom 96-well plate. The cells were incubated with 100 µL of Screen Quest™ Fura-2 No Wash Calcium Assay Kit for 1 hour at room temperature. ATP (50 µL/well) was added by a FlexStation (Molecular Devices) to achieve the final indicated concentrations.

DISCLAIMER

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