ab176766
Calcium Flux Assay Kit
(Fura-2, No Wash, Ratiometric)

Instructions for use:

For the measurement of intracellular calcium mobilization in live cells.

This product is for research use only and is not intended for diagnostic use.

Version 4d Last Updated 04 July 2025

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1. BACKGROUND

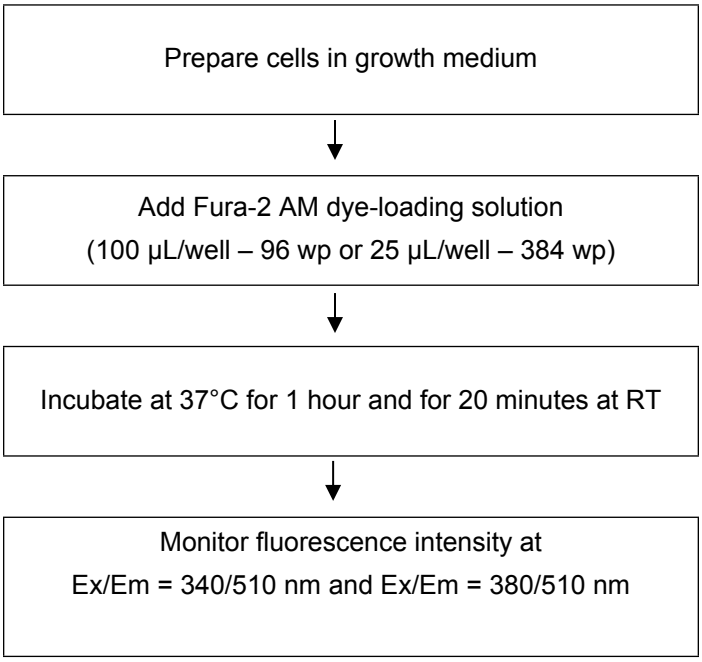
Abcam's Calcium Flux Assay Kit (Fura-2, No Wash, Ratiometric) (ab176766) is a ratiometric calcium assay that allows homogenous measurement of intracellular calcium mobilization caused by activation of G-protein coupled receptors (GPCR) or calcium channels. The cell-permeable dye Fura-2 AM is loaded into the culture media of cells expressing a GPCR of interest that signals through calcium. The dye can easily cross the cell membrane, and once inside the cell, the lipophilic blocking groups of Fura-2 AM are cleaved by non-specific cell esterases, creating a negatively charged fluorescent dye which is sequestered inside the cell. The assay does not require washing step and can be performed in a convenient 96-well or 384-well microtiter plate format.

The dual excitation ratio at 340/380 nm allows accurate measurement of intracellular calcium (Ca2+) concentration. Key features of ratio measurement:

- Reduced effects of uneven dye loading and cell numbers
- Reduced dye leakage
- Reduced photobleaching

This product has been optimized for HTS screening. We recommend to use this product with the following imaging plate readers: FLIPR™, FDSS, BMG NOVOstar™, FlexStation, ViewLux, IN Cell Analyzer or Arrayscan.

2. ASSAY SUMMARY



INTRODUCTION

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. MATERIALS SUPPLIED

Item	Amount		Storage Condition (Before Preparation)	Storage Condition (After Preparation)
	10 Plates	100 Plates		
Fura-2 AM	1 Vial	10 x 1 Vial	-20°C	-20°C
HHBS	100 mL	-	-20°C	-20°C
10X Pluronic® F127 Plus	10 x 1 mL	10 x 10 mL	-20°C	-20°C

7. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- HTS fluorescence microplate reader with inside dispenser to add the compounds to cell plate. We recommend instruments from the following manufacturers: FLIPR™, FDSS, BMG NOVOstar™, FlexStation, ViewLux, IN Cell Analyzer or Arrayscan
- MilliQ water or other type of double distilled water (ddH_2O)
- Sterile PBS
- 100% DMSO
- HHBS (1X Hank's with 20 mM Hepes Buffer, pH 7.0)
- General tissue culture supplies
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- Sterile, tissue culture treated, 96 or 384 well plate with clear flat bottom, preferably black. For suspension cells, plate should be coated with poly-D lysine prior cell addition.
- (Optional) Carbachol (ab141354) or other GPCR agonists to induce agonist-mediated calcium release in cells.

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples which generate values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening

9.1. **Fura-2 AM (lyophilized):**

Prepare a Fura-2 AM stock solution by adding 200 µL of DMSO into a single vial of lyophilized dye, and mix well by pipetting up and down.

Only 20 µL of reconstituted Fura-2 AM stock solution are necessary for 1 x 96 well plate. Aliquot unused reconstituted stock solution so that you have enough volume to perform the desired number of assays. Avoid repeated freeze/thaw. Store at -20°C in the dark. Reconstituted Fura-2 is stable for up to three months.

9.2. **HHBS:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

NOTE: *HHBS is not included in the 100 x 96 tests size.*

9.3. **10X Pluronic® F127 Plus:**

Dilute the 10X Pluronic® F127 Plus to prepare a 1X Assay Buffer: into a bottle containing 1 mL 10X Pluronic® F127 Plus, add 9 mL of HHBS Buffer (section 9.2) and mix well by inversion. Label this bottle as "**1X Assay Buffer**".

NOTE: *for 100 x 96 tests kit – add whole bottle of 10 mL to 90 mL of HHBS buffer and mix well.*

Only 10 mL of 1X Assay Buffer are necessary for 1 x 96 well plate. Aliquot unused 1X Assay Buffer so that you have enough volume to perform the desired number of assays. Avoid repeated freeze/thaw. Store at -20°C in the dark.

10. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to correct temperature prior to use.**
- **We recommended to assay all controls and samples in duplicate.**
- **Prepare all reagents and samples as directed in the previous sections.**
- **Do not add additional probenecid to samples.**

10.1. **Fura-2AM dye loading solution (1 plate)**

Add 20 µL of Fura-2 AM stock solution (section 9.1) into 10 mL of 1X Assay Buffer (section 9.3) and mix well by gently shaking the bottle.

NOTE: *this dye-loading solution is stable for at least 2 hours at room temperature.*

10.2. **Calcium assay – Adherent cells**

10.2.1. Plate cells overnight in appropriate growth medium in a black well/clear bottom plate.

96-wp: 40,000 – 80,000 cells/well/100 µL.

384-wp: 10,000 – 20,000 cells/well/25 µL.

NOTE: *Each cell line should be evaluated on an individual basis to determine the optimal cell density for the intracellular calcium mobilization.*

10.2.2. Add 100 µL/well (96-wp) or 25 µL/well (384-wp) of Fura-2 AM loading solution (step 10.2) into the cell wells.

NOTE: *If your compounds interfere with the serum, it is important to replace the growth medium with HHBS Buffer (100 µL/well for a 96-well plate or 25 µL/well for a 384-well) plate before dye-loading.*

10.2.3. Incubate the plate at 37°C in a cell incubator for 1 hour.

10.2.4. Take plate from incubator and incubate for another 20 minutes at room temperature. The incubation at RT will avoid or decrease the "dip" originated by the compound addition due to the different temperature between the cell plate and the compound plate.

NOTE: *Some cell lines, such as cells expressing chemokine receptors, perform better at 37°C only. In this case, skip the incubation at room temperature.*

10.2.5. Prepare the compound plate by using HHBS or your desired buffer. Add solution to compound dispenser on your instrument.

10.2.6. Using your instrument that contains a pipettor (such as Flexstation from Molecular Devices), dispense compounds directly onto to the cell plate while collecting the data simultaneously (see next step for Ex/Em instructions).

NOTE: *Typically the instrument collects the data every second for 100 seconds. Use the max signal to generate the plot. You can use a fluorescence microscope by adding the stimuli while taking the picture simultaneously.*

10.2.7. Run the calcium flux assay by monitoring the fluorescence increase at Ex/Em = 340/510 nm and Ex/Em = 380/510 nm as described in the instrument manuals.

NOTE: *Dispense speed and height for compound additions need to be optimized for each assay.*

For assays performed on a FlexStation, use the following wavelength parameters:

Excitation	Emission	Cut-off
Lm1 340	510 nm	455 nm
Lm2 380	510 nm	455 nm

For assays performed on a FDSS, use the standard filters for Fure-2 calcium assays on the instrument.

10.3. **Calcium assay – Suspension cells**

10.3.1. Grow suspension cells in appropriate growth medium.

10.3.2. Centrifuge cells and resuspend cell pellets in equal amounts of HHBS and Fura-2 AM dye loading solution (step 10.2).

10.3.3. Plate cells in a black well/clear bottom poly-D lysate plate:

96-wp: 125,000 – 250,000 cells/well/100 µL.

384-wp: 30,000 – 60,000 cells/well/25 µL.

10.3.4. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to the experiment.

NOTE: *Each cell line should be evaluated on an individual basis to determine the optimal cell density for the intracellular calcium mobilization.*

10.3.5. Incubate the plate at 37°C in a cell incubator for 1 hour.

10.3.6. Take plate from incubator and incubate for another 20 minutes at room temperature. The incubation at RT will avoid or decrease the “dip” originated by the compound addition due to the different temperature between the cell plate and the compound plate.

NOTE: Some cell lines, such as cells expressing chemokine receptors, perform better at 37°C only. In this case, skip the incubation at room temperature.

10.3.7. Prepare the compound plate by using HHBS or your desired buffer. Add solution to compound dispenser on your instrument.

10.3.8. Using your instrument that contains a pipettor (such as Flexstation from Molecular Devices), dispense compounds directly onto to the cell plate while collecting the data simultaneously (see next step for Ex/Em instructions).

NOTE: *Typically the instrument collects the data every second for 100 seconds. Use the max signal to generate the plot. You can use a fluorescence microscope by adding the stimuli while taking the picture simultaneously.*

10.3.9. Run the calcium flux assay by monitoring the fluorescence increase at Ex/Em = 340/510 nm and Ex/Em = 380/510 nm as described in the instrument manuals.

NOTE: *Dispense speed and height for compound additions need to be optimized for each assay.*

For assays performed on a FlexStation, use the following wavelength parameters:

Excitation	Emission	Cut-off
Lm1 340	510 nm	455 nm
Lm2 380	510 nm	455 nm

For assays performed on a FDSS, use the standard filters for Fure-2 calcium assays on the instrument.

11. CALCULATIONS

- Data can be presented as % Control based on the fluorescence ratio of 340/380 nm. If using FlexStation, the software provided has the program to do the calculation incorporated.

12. TYPICAL DATA

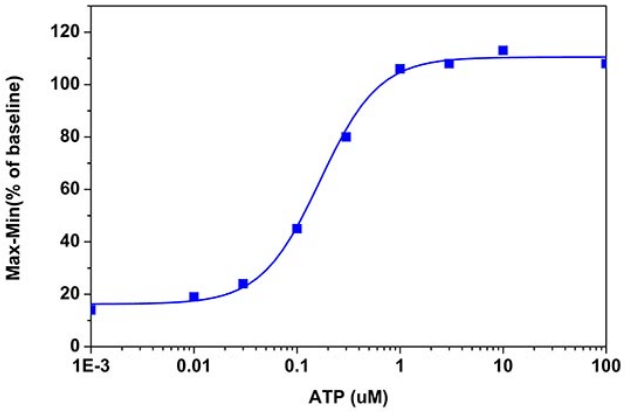


Figure 1: ATP dose response in CHO-K1 cells measured with Calcium Flux Assay Kit (Fura-2, No Wash, Ratiometric) (ab176766). CHO-K1 cells were seeded overnight at 40,000 cells/100 µL/well in a Costar black wall/clear bottom 96-well plate. The cells were incubated with 100 µL of Fura-2 AM loading solution for 1 hour at room temperature. ATP (50 µL/well) was added by a FlexStation (Molecular Devices) to achieve the final indicated concentrations.

13. QUICK ASSAY PROCEDURE

NOTE: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare Fura-2 AM stock solution and make 1X Assay Buffer (aliquot unused material); get equipment ready.
- Make Fura-2 AM dye-loading solution for one plate: 20 µL Fura-2 AM stock + 10 mL of 1X Assay Buffer.
- Set up cells in growth medium in a 96-wp (100 µL) or in a 384-wp (25 µL) with black wall and clear flat bottom.
- Add 100 µL/well (96-wp) or 25 µL/well (384-wp) of Fura-2 AM dye-loading solution to cells.
- Incubate plate at 37°C for 1 hour + 20 minutes incubation at RT
- Prepare compound plate with HHBS or desired buffer.
- Monitor fluorescence increase at Ex/Em = 340/510 nm and Ex/Em = 380/510 nm as described in your instrument manuals.

14. FAQ

Is probenecid included in any of the reagents? If so, what is the final concentration?

Yes, the 10X Pluronic® F127 Plus buffer contains 50 mM of probenecid. The working concentration is 5 mM and therefore the final concentration in the assay is 2.5 mM.

15. NOTES

RESOURCES

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)