Version 1 Last updated 9 April 2018

ab233472
Calcium Flux Assay Kit (Flow cytometry)

For the measurement of intracellular calcium mobilization using a flow cytometer.

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

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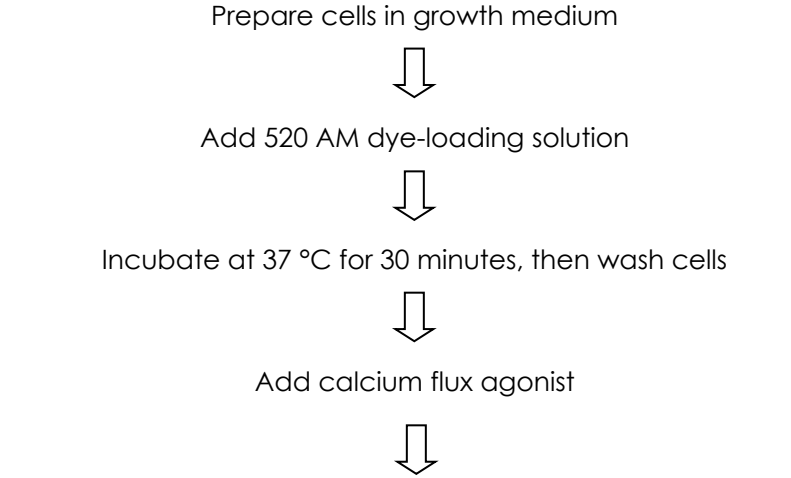
1. Overview

Calcium Flux Assay Kit (Flow cytometry) (ab233472) is a fluorescence-based assay for detecting intracellular calcium mobilization using a flow cytometer. It can be used for kinetic reading or for endpoint reading.

After loading the 520 AM dye into the cells of interest, simply wash the cells and add the calcium flux agonist then read the sample via a flow cytometer using kinetic reading mode or endpoint reading mode at Ex/Em = 490/525 nm.



2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

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

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
520 AM dye	1 vial	-20°C	-20°C
Assay Buffer	50 mL	-20°C	-20°C
25 mM Probenecid	3 mL	-20°C	-20°C
HHBS (with 20 mM HEPES)	100 mL	-20°C	-20°C

3. Materials Required, Not Supplied

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

- DMSO
- 0.5 mM EDTA
- Flow cytometer capable of reading at Ex/Em = 490/525 nm

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 520 AM dye

Prepare just before running the assay. Add 100 µL of DMSO into the vial of 520 AM dye and mix well.

ΔNote: *100 µL of 520 AM dye stock solution is enough for 100 assays. Unused 520 AM dye 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.*

5.2 Assay Buffer

Ready to use as supplied.

5.3 25 mM Probenecid

Ready to use as supplied. Dilute if needed.

5.4 HHBS (with 20 mM HEPES)

Ready to use as supplied.

6. Assay Procedure

6.1 Prepare cells:

1. For non-adherent cells, prepare cells in 0.5 mL cell in Assay Buffer at the density of $1x10^6$ – $2x10^6$ cells/mL.
2. For adherent cells, plate cells at $4x10^5$ - $8x10^5$ cells/mL in cell growth medium the day before experiment. On experiment day, the cells should be confluent. Remove the cell medium and add 0.5 mL of Assay Buffer.

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

6.2 Run calcium assay:

1. Prepare 520 AM dye stock solution (Step 5.1).

2. Add 1µL 520 AM stock solution (from Step 6.2.1) into 0.5 mL non-adherent or adherent cells in Assay Buffer.

   Δ Note: *If your cells (such as CHO cells) contain organic anion-transports, then Probenecid may be added to the 520 AM dye working solution (final in well concentration will be 0.125-1 mM) to reduce leakage of the de-esterified indicators. For example, use 0.125 mM Probenecid by adding 2.5 µL Probenecid into 500 µL cells in Assay Buffer with 1 µL 520 AM dye in CHO-K1 cells.*

3. Incubate the cells in a cell incubator for 30 minutes.

4. For non-adherent cells, centrifuge the cells and remove the dye. Re-suspend the cells in 0.4 mL HHBS (with 20 mM HEPES). For adherent cells, use 0.5 mM EDTA to gently lift the cells from the plate and centrifuge. Re-suspend the cells in 0.4 mL HHBS (with 20 mM HEPES).

   Δ Note: *For detaching adherent cells from the plate, enzymatic reagents (e.g. trypsin) can be considered but need to be tested to make sure the receptor of interest on the cell surface is not affected.*

5. Prepare 5X agonist compound with HHBS or your desired buffer.
6. Analyze the sample before and after the addition of 100 µL of the prepared agonist from step 6.2.5 on a flow cytometer at Ex/Em = 490/525 nm.

   Δ Note: *To achieve the best results, it is important to run the assay within 1 minute after the addition of the agonist. It is also important to make sure the time between the agonist addition and the beginning of the actual reading stays constant for all the samples.*

7. Typical Data

Data provided **for demonstration purposes only.**

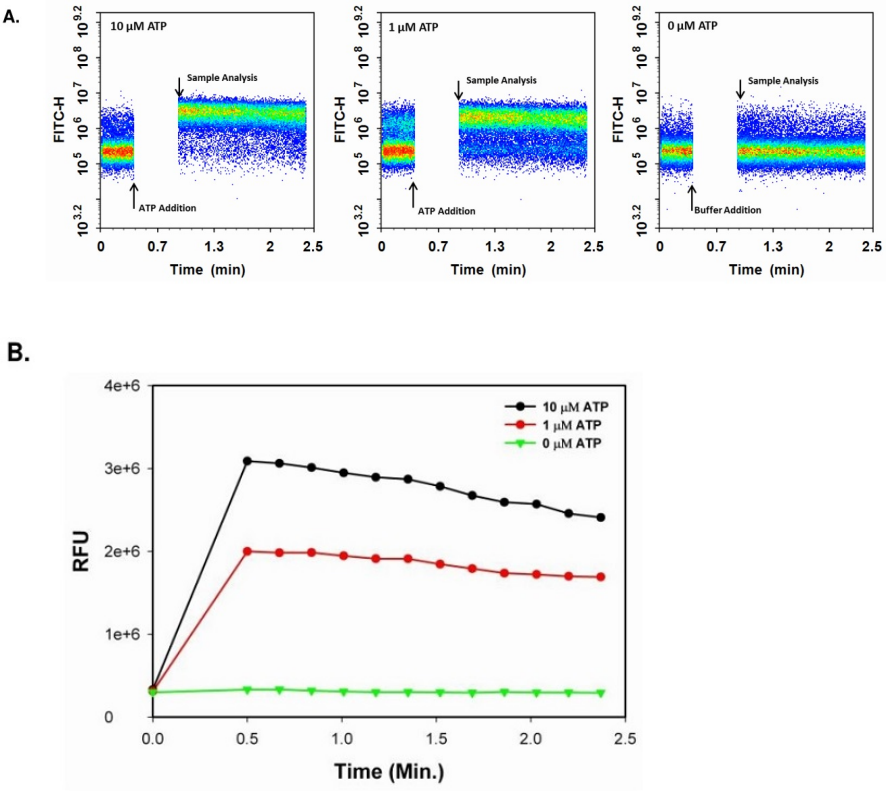


Figure 1. The ATP dose dependent intracellular calcium release was measured by Calcuim Flux Assay Kit (Flow cytometry) (ab233472) in CHO-K1 cells. Cells were incubated with 520 AM dye for 30 minutes at 37 °C before ATP was added into the cells. The baseline was acquired and the rest of the cells were analyzed after the addition of ATP. The response was measured over time. The analysis was done on NovoCyte™ 3000 Flow Cytometer.

A. 10 µM, 1 µM or 0 µM ATP were added to the cells. The arrows on the graph indicate the time (30 seconds) between addition of ATP and the actual analysis.

B. Time- dependent changes of fluorescent signal.

8. Notes

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