Cell Meter™ Flow Cytometric Calcium Assay Kit

Catalog number: 36310
Unit size: 100 Tests

Component	Storage	Amount
Component A: Calbryte™ 520 AM	Freeze (<-15 °C), Minimize light exposure	1 vial, lyophilized
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (50 mL)
Component C: Probenecid (Optional)	Freeze (<-15 °C), Minimize light exposure	1 bottle (3 mL, 25mM)
Component D: HHBS (Hanks' with 20 mM Hepes)	Refrigerate (2-8 °C)	1 bottle (100 mL)

OVERVIEW

Cell Meter™ Flow Cytometric Calcium Assay Kit provides fluorescence-based assays for detecting intracellular calcium mobilization using a flow cytometer. It can be used for kinetic reading or for endpoint reading. After loading the Calbryte™ 520 AM dye into cells of interest, simply wash the cells and add the calcium flux agonist, one can then read the sample via a flow cytometer using kinetic reading mode or endpoint reading mode. Calbryte™ 520 AM can cross cell membrane passively by diffusion. Once inside the cells, the lipophilic blocking groups of Calbryte™ 520 AM are cleaved by esterase, resulting in a negatively charged fluorescent dye that stays inside cells. Its fluorescence is greatly enhanced upon binding to calcium. When cells expressing GPCR of interest are stimulated with an agonist, the receptor signals the release of intracellular calcium, which significantly increases the fluorescence of Calbryte™ 520. The Cell Meter™ Flow Cytometric Calcium Assay Kit can be used for monitoring cellular calcium flux as well as cell sorting.

AT A GLANCE

Protocol summary

1. Prepare cells in Assay Buffer
2. Add Calbryte™ 520 AM dye-loading solution (1 µL)
3. Incubate at 37°C for 30 minutes
4. Wash the cells
5. Add calcium flux stimulator
6. Monitor fluorescence intensity with flow cytometer using 530/30 nm filter (FITC channel)

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

KEY PARAMETERS

Instrument:	Flow cytometer
Excitation:	488 nm laser
Emission:	530/30 nm filter
Instrument specification(s):	FITC channel

Other Instruments: FDSS, NOVOStar, FlexStation, ViewLux, IN Cell Analyzer, ArrayScan

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.

Calbryte™ 520 AM stock solution (500X):
Add 100 µL of DMSO (Not provided) into the vial of Calbryte™ 520 AM stock solution (Component A) and mix them well.

Note 100 µL of Calbryte™ 520 AM stock solution is enough for 100 assays. Unused Calbryte™ 520 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.

SAMPLE EXPERIMENTAL PROTOCOL

1. Remove cell culture medium and add 0.5 mL of Assay Buffer.

   Note For adherent cells and non-adherent cells, 4 X10^5 – 8 X10^5 and 1X10^6 - 2X10^6 are recommended to use, respectively. Each cell line should be evaluated on the individual basis to determine the optimal cell density for the intracellular calcium mobilization.

2. Add 1 µL Calbryte™ 520 AM stock solution (500X) into 0.5 mL non-adherent or adherent cells in Assay Buffer (Component B).

   Note If your cells (such as CHO cells) contain organic anion-transports, then probenecid (Component C) may be added to the dye working solution (final in well concentration will be 0.125-1 mM) to reduce leakage of the de-esterified indicators.

3. Incubate the cells at 37°C for 30 minutes.

4. For non-adherent cells, centrifuge the cells and remove the dye. Re-suspend the cells in 0.4 mL HHBS (Component D). 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 (Component D).

   Note For detaching adherent cells from the plate, enzymatic reagents (e.g. trypsin, Accutase) 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 on a flow cytometer using 530/30 nm filter (FITC channel).

   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.

EXAMPLE DATA ANALYSIS AND FIGURES

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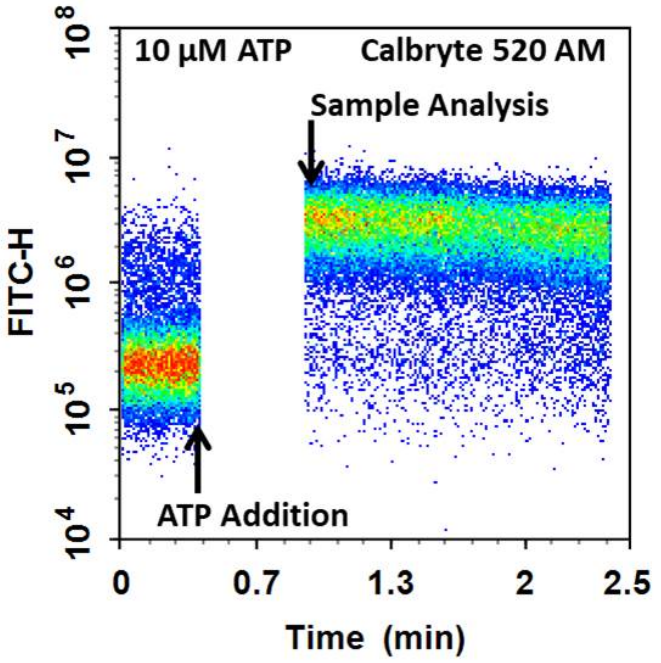

Figure 1.

The ATP induced intracellular calcium release was measured by Cell Meter™ Flow Cytometric Calcium Assay Kit in CHO-K1 cells. Cells were incubated with Calbryte™ 520 AM dye for 30 min at 37 °C before 10 µM 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. The arrows on the graph indicate the time (30 sec) between addition of ATP and the actual analysis.

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