Cell Meter™ Flow Cytometric Calcium Assay Kit

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 36310 (100 assays)	Keep in freezer and avoid light	Flow Cytometer

Introduction

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 at Ex/Em = 490/525 nm. 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.

Kit Components

Components	**Amount**
Component A: Calbryte™ 520 AM	1 Vial, lyophilized
Component B: Assay Buffer	1 Bottle (50 mL)
Component C: Probenecid (Optional)	1 Bottle (3 mL, 25 mM)
Component D: HHBS (Hanks' with 20 mM Hepes)	1 Bottle (100 mL)

Assay Protocol for Flow Cytometer

Brief Summary

Prepare cells in growth medium → Add Calbryte™ 520 AM dye-loading solution → Incubate at 37 °C for 30 minutes → Wash the cells → Add calcium flux stimulator → Monitor fluorescence at Ex/Em = 490/525 nm on Flow Cytometer

Note: Thaw all the kit components at room temperature before use.

1. **Prepare cells:**

1.1. For non-adherent cells, prepare cells in 0.5 mL cell in Assay Buffer (Component B) at the density of $1X10^6$ - $2X\ 10^6$ cells/mL.

1.2. For adherent cells, plate cells at $4\ X10^5$ – $8\ X10^5$ 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 (Component B).

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

2. **Prepare 500X Calbryte™ 520 AM stock solution**: Add 100 µL of DMSO into one vial of Calbryte™ 520 AM (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.

3. **Run calcium assay**:

3.1 Add 1µL 500X Calbryte™ 520 AM stock solution (from Step 2) 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-

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esterified indicators. We have used 0.125 mM probenecid by adding 2.5 µL of Component C into 500 µL cells in Assay Buffer with 1 µL Calbryte™ 520 AM dye in CHO-K1 cells.

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

3.3 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.

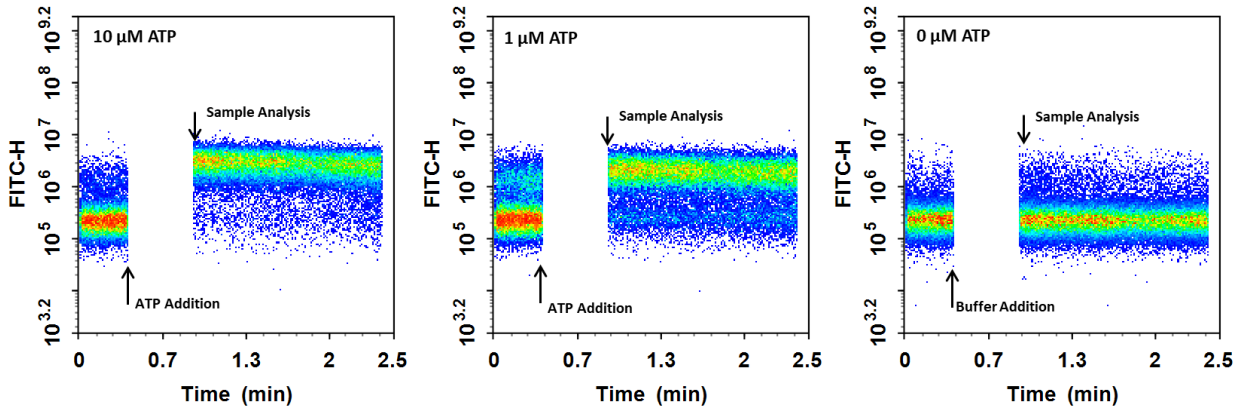
3.4 Prepare 5X agonist compound with HHBS or your desired buffer.

3.5 Analyze the sample before and after the addition of 100 µL of the prepared agonist from step 3.4 on a flow cytometer at Ex/Em = 490/525 nm.

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.

Data Analysis

A.



B.

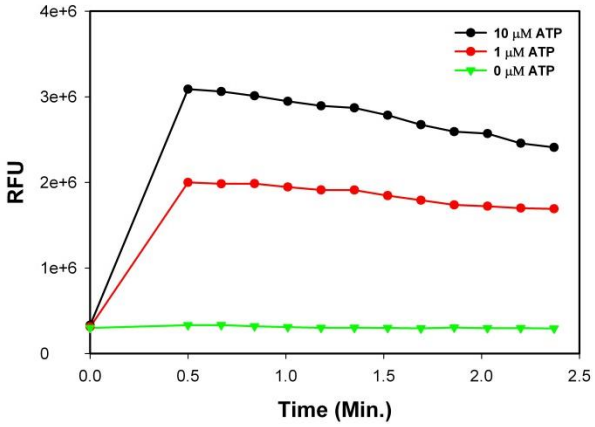


Figure 1. The ATP dose dependent intracellular calcium release was measured by Cell Meter™ Flow Cytometric Calcium Assay Kit in CHO-K1 cells. Cells were incubated with Calbryte™ 520 AM dye for 30 min at 37 °C before ATP was added into the cells. The baseline was acquired and the rest of the cells were 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 sec) between addition of ATP and the actual analysis. **B**. Time-dependent changes of fluorescent signal.

References:

1. Berridge, M.J., Lipp, P., Bootman, M.D., 2000. The versatility and universality of calcium signaling. Nat. Rev. Mol. Cell Biol. 1 (1), 11.
2. Wendt, ER., Ferry H., Greaves, DR and Keshav S., 2015. Ratiometric Analysis of Fura Red by Flow Cytometry: A Technique for Monitoring Intracellular Calcium Flux in Primary Cell Subsets. PLOS One.