Screen Quest™ Calbryte-520 Probenecid-Free and Wash-Free Calcium Assay Kit

Catalog number: 36317, 36318, 36319
Unit size: 1 Plate, 10 Plates, 100 Plates

Component	Storage	Amount (Cat No. 36317)	Amount (Cat No. 36318)	Amount (Cat No. 36319)
Component A: Calbryte™ 520 AM	Freeze (< -15 °C), Minimize light exposure	1 vial	1 vial	10 vials
Component B: 10X Pluronic® F127 Plus	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mL)		
Component B: 10X Pluronic® F127 Plus	Freeze (< -15 °C), Minimize light exposure		1 bottle (10 mL)	10 bottles (10 mL/bottle)
Component C: HHBS	Freeze (< -15 °C), Minimize light exposure		1 bottle (100 mL)	Not provided
Component C: HHBS (Hanks' buffer with 20 mM Hepes)	Freeze (< -15 °C), Minimize light exposure	1 vial (9 mL)	1 bottle (100 mL)	

OVERVIEW

Screen Quest™ Calbryte™ 520 Probenecid-Free and Wash-Free Calcium Assay Kit is designed for fluorescence-based detection of intracellular calcium mobilization in live cells. Calcium flux assays are widely used in drug discovery for screening G protein-coupled receptors (GPCRs) and calcium channels. The kit uses Calbryte™ 520 AM, a cell-permeant calcium indicator that enters cells and is converted by intracellular esterases into a calcium-responsive fluorescent dye. The Calbryte™ 520 dye is designed for improved cellular retention, enabling calcium flux detection without probenecid and without wash steps. When cells are stimulated with screening compounds, receptor-mediated calcium release or calcium influx produces an increase in green fluorescence that can be measured in a microplate format. The homogeneous assay workflow is compatible with 96-well and 384-well formats and can be adapted to automated screening systems. Screen Quest™ Calbryte™ 520 Probenecid-Free and Wash-Free Calcium Assay Kit is suitable for GPCR calcium signaling studies, calcium channel assays, high-throughput screening, and calcium flux analysis in fragile or difficult cell lines.

AT A GLANCE

Protocol Summary

1. Prepare cells in growth medium
2. Add Calbryte™ 520 AM dye-loading solution (100 µL/well for 96-well plate or 25 µL/well for 384-well plate)
3. Incubate at room temperature or 37 °C for 30-60 minutes
4. Monitor fluorescence at Ex/Em = 490/525 nm

Important Note

Thaw all the kit components at room temperature before use.

KEY PARAMETERS

Fluorescence microplate reader

Excitation	490 nm
Emission	525 nm
Cutoff	515 nm
Recommended plate	Black wall/clear bottom
Instrument specification(s)	Bottom read mode/Programmable liquid handling

Other instruments: FDSS, FLIPR, ViewLux, NOVOStar, ArrayScan, FlexStation, IN Cell Analyzer

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

Calbryte™ 520 AM stock solution

Add 20 µL (**Cat. # 36317**) or 200 µL (**Cat. # 36318 and # 36319**) of DMSO into the vial of Calbryte™ 520 AM (Component A) and mix them well.

Note: 20 µL of Calbryte™ 520 AM stock solution is enough for one plate. 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.

Note: Protect from light and avoid repeated freeze-thaw cycles.

Assay buffer (1X)

Mix 9 mL of HHBS (Component C, not included in the kit **Cat. # 36319**) with 1 mL of 10X Pluronic® F127 Plus (10X) (Component B) and mix them well.

PREPARATION OF WORKING SOLUTION

Calbryte™ 520 AM dye-loading solution

Add 20 µL of Calbryte™ 520 AM stock solution into 10 mL of Assay Buffer (1X) and mix them well.

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

Note: 10 mL dye-loading solution is enough for one 96-wells plate.

SAMPLE EXPERIMENTAL PROTOCOL

1. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of Calbryte™ 520 AM dye-loading solution into the cell plate.

2. Incubate the dye-loading plate in a cell incubator for 30-60 minutes, and then incubate the plate at room temperature for another 15-30 minutes. *Note:* If the assay requires 37 °C, perform

the experiment immediately without further room temperature incubation. If the cells can function well at room temperature for longer time, incubate the cell plate at room temperature for 1 hour (It is recommended that the incubation time be no longer than 2 hours.)

Note: Do NOT wash the cells after dye loading.

3. Prepare the compound plate with HHBS or your desired buffer.
4. Run the calcium flux assay by monitoring the fluorescence intensity at Ex/Em = 490/525 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate ATP samples. We recommend using the Online Four Parameter Logistics Calculator which can be found at:

https://www.aatbio.com/tools/four-parameter-logistic-4pl-curve-regression-online-calculator

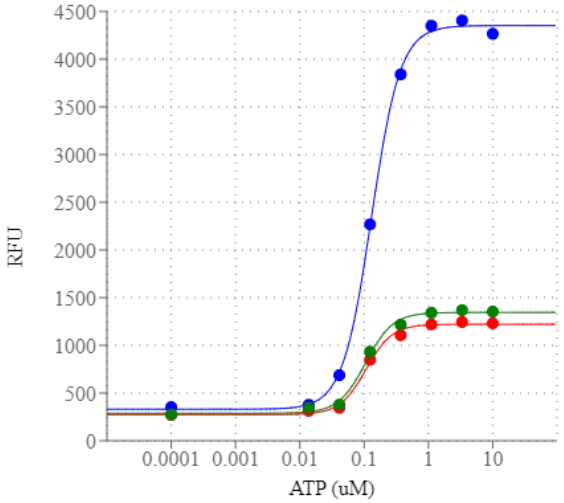

Figure 1. Comparison of fluorescent signal response of endogenous P2Y receptor to ATP in CHO-K1 cells. CHO-K1 cells were seeded overnight at 50,000 cells/100 µL/well in a 96-well black wall/clear bottom costar plate. Calcium flux response was measured with Screen Quest™ Calbryte™ 520 Probenecid-Free and Wash-Free Calcium Assay Kit, FLIPR Calcium 4 Assay Kit , and Fluo-4 Direct Calcium Assay kit. ATP (50 µL/well) was added by FlexStation 3 to achieve the final indicated concentrations.

DISCLAIMER

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