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)	1 bottle (10 mL)	10 bottles (10 mL/bottle)
Component C: HHBS (Hanks' buffer with 20 mM Hepes)	Freeze (< -15 °C), Minimize light exposure	1 vial (9 mL)	1 bottle (100 mL)	Not provided

OVERVIEW

Calcium flux assays are the preferred methods in drug discovery for screening G protein coupled receptors (GPCR). Screen Quest™ Calbryte-520 Probenecid-Free and Wash-Free Calcium Assay Kit provides the most robust homogeneous fluorescence-based assay for detecting the intracellular calcium mobilization. Cells expressing a GPCR of interest that signals through calcium are pre-loaded with our proprietary Calbryte™-520 AM which can cross cell membrane. Calbryte™-520 AM is the brightest calcium indicator available for HTS screening. Once inside the cell, the lipophilic blocking groups of Calbryte™-520 AM are cleaved by non-specific cell esterase, resulting in a negatively charged fluorescent dye that stays inside cells, and its fluorescence is greatly enhanced upon binding to calcium. When cells stimulated with screening compounds, the receptor signals release of intracellular calcium, which greatly increase the fluorescence of Calbryte™-520 AM. The characteristics of its excellent cell retention, high sensitivity, and 100-250 times fluorescence increases (when it forms complexes with calcium) make Calbryte™-520 AM an ideal indicator for measurement of cellular calcium. Calbryte™-520 AM is the only calcium dye that does not require probenecid for better cellular retention. This Screen Quest™ Calbryte-520 Probenecid-Free and Wash-Free Calcium Assay Kit provides the most optimized assay method for monitoringG-protein-coupled receptors (GPCRs) and calcium channels with fragile or difficult cell lines. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation.

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 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.

1. 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.

2. 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

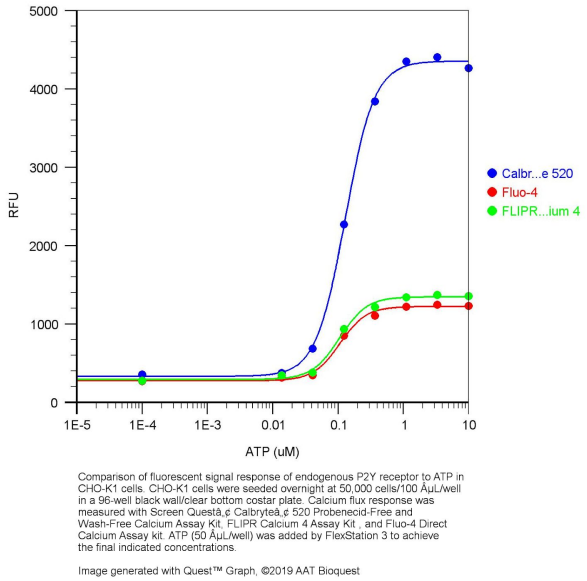

Figure 1. Comparison of fluorescent signal response of endogenous P2Y receptor to ATP in CHO-K1 cells. CHO-K1 cells were seeded overnight at 50,000 cells/100 µL/well in a 96-well black wall/clear bottom costar plate. Calcium flux response was measured with Screen Quest™ Calbryte™ 520 Probenecid-Free 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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