Screen Quest™ Calbryte-590 Probenecid-Free and Wash-Free Calcium Assay Kit

Catalog number: 36200, 36201, 36202
Unit size: 1 Plate, 10 Plates, 100 Plates

Component	Storage	Amount		
		Cat No. 36200	Cat No. 36201	Cat No. 36202
Component A: Calbryte™ 590 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 bottle (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 bottle (9 mL)	1 bottle (100 mL)	Not provided

OVERVIEW

Calcium flux assays are the preferred methods for screening G protein coupled receptors (GPCRs) in drug discovery. Screen Quest™ Calbryte-590™ Probenecid-Free and Wash-Free Calcium Assay Kit provides the most robust homogeneous red 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-590 AM which can cross cell membrane. Calbryte-590 AM is the brightest calcium indicator available for HTS screening. Once inside the cell, the lipophilic blocking groups of Calbryte-590 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-590 AM. The characteristics of its excellent cell retention, high sensitivity, and 100-250 times fluorescence increases (when it forms complexes with calcium) make Calbryte-590 AM an ideal indicator for measurement of cellular calcium. Calbryte-590 AM is the only red calcium dye that does not require probenecid to improve cellular retention. This Screen Quest™ Calbryte-590™ Probenecid-Free and Wash-Free Calcium Assay Kit provides the most optimized assay method for monitoring GPCRs and calcium channels with fragile or difficult cell lines. Compared to the green fluorescence-based calcium assays, this assay kit has less interference from the colored compounds from a compound library. It is also compatible with GFP cell lines for high content analysis. 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™ 590 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 45-60 minutes
4. Monitor fluorescence at Ex/Em = 540/590 nm

Important Thaw all the kit components at room temperature before use.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 nm
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	Bottom read mode/programmable liquid handling

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.

1. Calbryte™ 590 AM stock solution:
Add 20 µL (**Cat. # 36200**) or 200 µL (**Cat. # 36201 and # 36202**) of DMSO into the vial of Calbryte™ 590 AM (Component A) and mix them well.

Note 20 µL of Calbryte™ 590 AM stock solution is enough for one plate. Unused Calbryte™ 590 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. # 36202**) with 1 mL of 10X Pluronic® F127 Plus (10X) (Component B) and mix them well.

PREPARATION OF WORKING SOLUTION

Calbryte™ 590 AM dye-loading solution:
Add 20 µL of Calbryte™ 590 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.

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

1. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of Calbryte™ 590 AM dye-loading solution into the cell plate.

2. Incubate the dye-loading plate in a cell incubator for 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.)

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 = 540/590 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (SNR x 100%) 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.

Tel: 408-733-1055 | Fax: 408-733-1304 | Email: support@aatbio.com

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

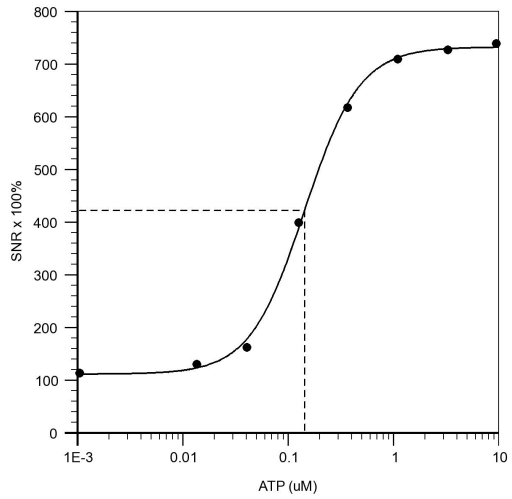

Figure 1. Graph illustrates signal-to-noise ratio (SNR) x 100%. ATP dose response was measured in CHO-K1 cells with Screen Quest™ Calbryte 590 Probenecid-Free and Wash-Free Calcium Assay Kit. CHO-K1 cells were seeded overnight at 50,000 cells/100 µL/well in a 96-well black wall/clear bottom costar plate. 100 µL dye loading solution was added and incubated for 45 min at 37°C and 15 min at RT. ATP (50 µL/well) was added by FlexStation 3 to achieve the final indicated concentrations.