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

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 36317(1 plate), 36318 (10 plates), 36319 (100 plates)	Keep in freezer and avoid light	FLIPR, FDSS, NOVOStar,FlexStation, ViewLux, IN Cell Analyzer, ArrayScan

Introduction

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-520NW which can cross cell membrane. Calbryte-520 NW is the brightest calcium indicator available for HTS screening. Once inside the cell, the lipophilic blocking groups of Calbryte-520NW 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-520NW. The characteristics of its excellent cell retention, high sensitivity, and 100-250 times fluorescence increases (when it forms complexes with calcium) make Calbryte-520NW an ideal indicator for measurement of cellular calcium. Calbryte-520NW 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 monitoring G-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.

Kit Components

Components	**36317 (1 plate)**	**36318 (10 plates)**	**36319 (100 plates)**
Component A: Calbryte™ 520NW	1 vial, lyophilized	1 vial, lyophilized	10 vials, lyophilized
Component B: 10X Pluronic F127 Plus	1 bottle (1 mL)	1 bottle (10 mL)	10 bottles (10 mL/bottle)
Component C: HHBS (Hanks' buffer with 20 mM Hepes)	1 bottle (9 mL)	1 bottle (100 mL)	Not provided

Assay Protocol for One Plate

Brief Summary

Prepare cells in growth medium → Add Calbryte™ 520NW dye-loading solution (100 µL/well/96-well plate or 25 µL/well/384-well plate) → Incubate at RT or 37°C → Monitor fluorescence at Ex/Em = 490/525 nm

1. Prepare cells:

1.1 For adherent cells: Plate cells overnight in growth medium at 40,000 to 80,000 cells/well/100 µL for a 96-well plate or 10,000 to 20,000 cells/well/25 µL for a 384-well plate.

1.2 For non-adherent cells: Centrifuge the cells from the culture medium and then suspend the cell pellet in Cell growth medium or HHBS at 125,000 to 250,000 cells/well/100 µL for a 96-well poly-D lysine plate or 30,000 to 60,000 cells/well/25 µL for a 384-well poly-D lysine plate. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to the experiments.
Note: Each cell line should be evaluated on the individual basis to determine the optimal cell density for the intracellular calcium mobilization.

2. Prepare Calbryte™ 520NW dye-loading solution:

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

2.2 Make Calbryte™ 520NW stock solution: Add 20 µL (for Cat. # 36317) or 200 µL (for Cat. # 36318 and # 36319) of DMSO into the vial of Calbryte™ 520NW (Component A), and mix them well.
Note: 20 µL of Calbryte™ 520NW stock solution is enough for one plate. Unused Calbryte™ 520NW 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.

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

2.4 Make Calbryte™ 520NW dye-loading solution for one cell plate: Add 20 µL of Calbryte™ 520NW stock solution (from Step 2.2) into 10 mL of 1X assay buffer (from Step 2.3), and mix them well. This working solution is stable for at least 2 hours at room temperature.

3. Run calcium assay:

3.1 Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of Calbryte™ 520NW dye-loading solution (from Step 2.4) into the cell plate.

3.2 Incubate the dye-loading plate in a cell incubator for 30 minutes, and then incubate the plate at room temperature for another 15-30 minutes.
Note 1: If the assay requires 37 °C, perform the experiment immediately without further room temperature incubation.
Note 2: 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.)

3.3 Prepare the compound plate with HHBS or your desired buffer.

3.4 Run the calcium flux assay by monitoring the fluorescence intensity at Ex/Em = 490/ 525 nm.

Data Analysis

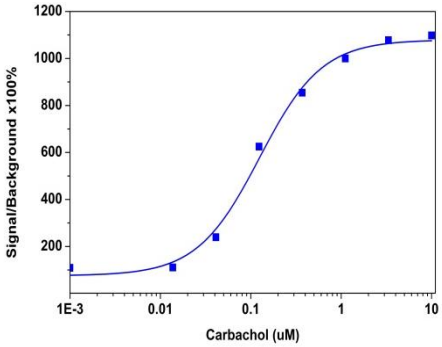

Figure 1. Carbachol dose response was measured in CHO-M1 cells with Screen Quest™ Calbryte™ 520 Probenecid-Free and Wash-Free Calcium Assay Kit. CHO-M1 cells were seeded overnight at 50,000 cells/100 µL/well in a 96-well black wall/clear bottom costar plate. 100 µL dye loading solution was added and incubated for 45 min at 37°C. Carbachol (50 µL/well) was added by FlexStation 3 to achieve the final indicated concentrations.

Warning: This kit is only sold to end users. It is patent-pending. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at info@aatbio.com if you have any questions.