Cal-520™ No Wash Calcium Assay Kit *Optimized for difficult cell lines*

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 36338(10 plates) 36339 (100 plates)	Freeze and avoid light	FLIPR, FDSS, NOVOStar,FlexStation, ViewLux, IN Cell Analyzer, ArrayScan

Introduction

Cal-520™ No Wash Calcium Assay Kits provide homogeneous fluorescence-based assays for detecting intracellular calcium mobilization. Cal-520™ AM is a new colorless fluorescent calcium-sensitive dye with a significantly improved signal to noise ratio and intracellular retention. Cells expressing a GPCR or calcium channel of interest that signals through calcium are pre-loaded with Cal-520™ AM which can cross cell membrane. Once inside the cell, the lipophilic blocking groups of Cal-520™ AM are cleaved by esterases, resulting in a negatively charged fluorescent dye that stays inside cells. Its fluorescence is greatly enhanced upon binding to calcium. When cells stimulated with agonists, the receptor signals the release of intracellular calcium, which significantly increase the fluorescence of Cal-520™. The characteristics of its long wavelength, high sensitivity, and >100 times fluorescence enhancement, make Cal-520™ AM an ideal indicator for the measurement of cellular calcium. The high S/B ratio and better intracellular retention make the Cal-520™ calcium assay a robust tool for evaluating GPCR and calcium channel targets as well as for screening their agonists and antagonists. Cal-520™ No Wash Calcium Assay Kits provide an optimized assay method for monitoring the GPCRs and calcium channels. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation.

Kit Key Features

Increased S/B Ratio:	Significantly higher signal/background ratio than any other fluorescent Ca^{2+}assays.
Convenient:	No wash and no probenecid needed. Formulated to have minimal hands-on time.
Versatile Applications:	Compatible with many cell lines and targets without ligand or target interference.

Kit Components

Components	**#36338 (10 plates)**	**#36339 (100 plates)**
Component A: Cal-520™, AM	1 vial, lyophilized	10 vials, lyophilized
Component B: 10X Pluronic F127 Plus	10 bottles (1 mL/bottle)	10 bottles (10 mL/bottle)
Component C: HHBS (Hanks' buffer with 20 mM Hepes)	1 bottle (100 mL)	Not included

Assay Protocol for One Plate

Brief Summary

Prepare cells in growth medium→ Add Cal-520™ dye-loading solution (100 µL/well for 96-well plate or 25 µL/well for 384-well plate)→ Incubate at 37°C for 1-2hours→ Read Fluorescence at Ex/Em= 490/525 nm

Caution: No addition of probenecid is needed.

1. Prepare Cells:

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

1.2 For non-adherent cells, centrifuge the cells from the culture medium and then suspend the cell pellets in equal amount of HHBS and Cal-520™ dye-loading solution (see steps 2.4 below) at 125,000 to 250,000 cells/well/100µL for 96-well or 30,000 to 60,000 cells/well/25µL for 384-well poly-D lysine plates. Centrifuge the plates at 800 rpm for 2 minutes with break off prior to the experiments

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

2. Prepare Cal-520™ dye-loading solution (for 1 plate):

2.1 Thaw 1 vial of Component A (Cal-520™, AM), 1 bottle of Component B (10X Pluronic F127 Plus) and Component C (HHBS) at room temperature before use.

2.2 Make Cal-520™ stock solution by adding 200 µL DMSO into Component A (Cal-520™, AM) and mixing them well.

*Note: **Cal-520™, AM is colorless**. 20 µL of reconstituted Cal-520™, AM is enough for 1 plate, unused Cal-520™ , AM can be aliquoted and stored at ≤-20°C for one month if the tubes are sealed tightly, avoided light and repeated freeze-thaw cycles.*

2.3 Make 1X assay buffer

a). For **Cat# 36338 (10 plates kit)**, make 1X assay buffer by adding **9 mL of** Component C (HHBS) into Component B (10X Pluronic F127 Plus, 1 mL), mix them well.

b).For **Cat# 36339(100 plates kit)**, make 1X assay buffer by adding whole bottle of Component B (10 X Pluronic F127 Plus, 10 mL) into **90 mL** HHBS buffer (not included in the kit), mix them well.

Note: 10 ml 1X assay buffer is enough for 1 plate, aliquot and store unused 1X assay buffer at ≤ -20°C, avoid light and repeated freeze-thaw cycles.

2.4 Make Cal-520™ dye-loading solution for one cell plate by adding 20 µL of DMSO reconstituted Cal-520™ (from Step 2.2) into 10 mL of 1X assay buffer (from Step 2.3), mixing 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) Cal-520™ dye-loading solution into the cell plate.
Note: If your compounds interfere with the serum, then it is important to replace the growth medium with HHBS buffer (100 µL/well for 96-well plate or 25 µL/well for 384-well plate before dye-loading). (We offer 2 separate medium removal calcium assay kits (#36336 and 36337) for the researchers who prefer the medium removal step).

3.2 Incubate the dye-loading plate at cell incubator for 60 to 90 minutes, and then incubate the plate at room temperature for another 30 minutes.
Note 1: Incubate the dye longer than 2 hours gives better signal intensity for some cell lines.
Note 2: If the assay requires 37°C, perform the experiment immediately without further room temperature incubation.

3.3 Prepare the compound plates by using HHBS or your desired buffer.

3.4 Run the calcium flux assay by monitoring the fluorescence at Ex/Em = 490/525 nm.
Note: It is important to run the signal test before your experiment. Different instruments have their own intensity range. Adjust the signal test intensity to the level of 5% to 10% of the maximum instrument intensity counts. For example, the maximum fluorescence intensity count for FLIPR-384 is 65,000, so the instrument settings should be adjusted to have its signal test intensity around 5,000 to 7,000.

Data Analysis

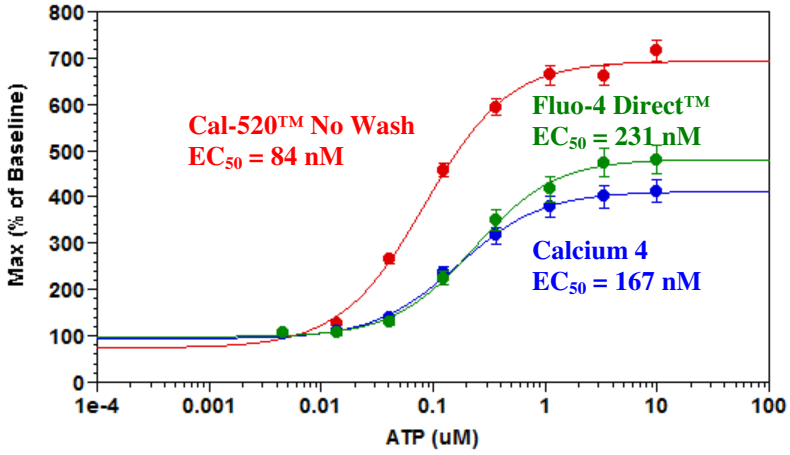

Figure1. **ATP dose response comparison in CHO-K1 cells measured with Cal-520™ No Wash, FLIPR Calcium 4 or Fluo-4 Direct™ Calcium Assay Kit.** CHO-K1cells were seeded overnight in 60,000 cells per 100 µL per well in a 96-well black wall/clear bottom costar plate. The cells were incubated with 100 µL of the Cal-520™ No Wash Calcium Assay Kit, FLIPR Calcium 4 Kit or Fluo-4 Direct™ Calcium Assay Kit (According to the Manufacturer's instructions) for 1.5 hours at 37°C. ATP (50µL/well) was added by FlexStation (Molecular Devices) to achieve the final indicated concentrations.