Calbryte™-520XL AM

Catalog number: 20646
Unit size: 10x50 ug

Component	Storage	Amount (Cat No. 20646)
Calbryte™-520XL AM	Freeze (< -15 °C), Minimize light exposure	10x50 ug

OVERVIEW

Calbryte™-520XL, AM is a new fluorescent and cell-permeable calcium indicator with extremely low affinity. Like other dye AM cell loading, Calbryte™-520XL AM ester is non-fluorescent, and once it gets inside the cell, it is hydrolyzed by intracellular esterase and gets activated. The activated indicator is a polar molecule that can no longer freely diffuse through the cell membrane, essentially trapped inside cells. Calbryte™-520XL has a low affinity for calcium ions with a Kd ~300 uM, similar to the well-known Rhod 5N, but it is much more stable. Calbryte™-520XL produces a bright fluorescence signal in the presence of calcium ions in high concentration. It has an excitation and emission wavelength identical to Fluo-4. Thus, the same Fluo-4 assay settings can be readily applied to Calbryte™-520XL-based calcium assays. Calbryte™-520XL is an excellent alternative to Rhod-5N. We also offer Calbryte™-520XL, potassium salt (#20645), Calbryte™-520XL-dextran (#20648), Calbryte™-520XL azide (#20643) that can be readily conjugated to a carrier through the well-known click chemistry.

KEY PARAMETERS

Fluorescence microscope

Emission	FITC
Excitation	FITC
Recommended plate	Black wall/clear bottom

Fluorescence microplate reader

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

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™-520XL AM Stock Solution

1. Prepare a 2 to 5 mM stock solution of Calbryte™-520XL AM in anhydrous DMSO.

   Note: The Calbryte™-520XL AM stock solution is a clear, colorless solution.

PREPARATION OF WORKING SOLUTION

Calbryte™-520XL AM Working Solution

1. On the day of the experiment, either dissolve Calbryte™ 520XL AM in DMSO or thaw an aliquot of the indicator stock solution to room temperature.

2. Prepare a 2 to 20 µM Calbryte™-520XL AM working solution in a buffer of your choice (e.g., Hanks and Hepes buffer) with 0.04% Pluronic® F-127. For most cell lines, Calbryte™ 520XL AM at a final concentration of 4-5 µM is recommended. The exact concentration of indicators required for cell loading must be determined empirically.

   Note: The nonionic detergent Pluronic® F-127 is sometimes used to increase the aqueous solubility of Calbryte™ 520XL AM. A variety of Pluronic® F-127 solutions can be purchased from AAT Bioquest.

   Note: If your cells contain organic anion-transporters, probenecid (1-2 mM) may be added to the dye working solution (final in well concentration will be 0.5-1 mM) to reduce leakage of the de-esterified indicators. A variety of ReadiUse™ Probenecid products, including water-soluble, sodium salt, and stabilized solutions, can be purchased from AAT Bioquest.

SAMPLE EXPERIMENTAL PROTOCOL

Following is our recommended protocol for loading AM esters into live cells. This protocol only provides a guideline and should be modified according to your specific needs.

1. Prepare cells in growth medium overnight.

2. On the next day, add 1X Calbryte™ 520XL AM working solution to your cell plate.

   Note: If your compound(s) interfere with the serum, replace the growth medium with fresh HHBS buffer before dye-loading.

3. Incubate the dye-loaded plate in a cell incubator at 37 °C for 30 to 60 minutes.

   Note: Incubating the dye for longer than 1 hour can improve signal intensities in certain cell lines.

4. Replace the dye working solution with HHBS or buffer of your choice (containing an anion transporter inhibitor, such as 1 mM probenecid, if applicable) to remove any excess probes.
5. Add the stimulant as desired and simultaneously measure fluorescence using either a fluorescence microscope equipped with a FITC filter set or a fluorescence plate reader containing a programmable liquid handling system such as an FDSS, FLIPR, or FlexStation, at Ex/Em = 490/525 nm cutoff 515 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

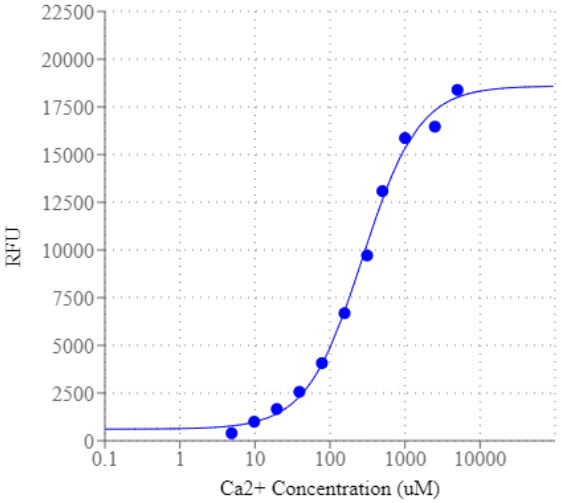

Figure 1. Ca2+ dependent fluorescence emission of Calbryte™-520XL indicator (Ex/Em = 490/525 nm).

DISCLAIMER

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