OG488 BAPTA-1, AM [equivalent to Oregon Green® 488 BAPTA-1, AM] *Cell permeant*

Catalog number: 20507
Unit size: 10x50 ug

Component	Storage	Amount
OG488 BAPTA-1, AM [equivalent to Oregon Green® 488 BAPTA-1, AM] *Cell permeant*	Freeze (< -15 °C), Minimize light exposure	10x50 ug

OVERVIEW

OG488 BAPTA -1 AM is the same molecule of Oregon Green 488 BAPTA-1 AM ester. It is a cell-permeable and visible light-excitable calcium indicator that is often used with FITC filter set. Cells may be loaded with OG488 BAPTA -1 AM by adding the dissolved indicator directly to dishes containing cultured cells. The fluorescence signal from these cells is generally measured using fluorescence microscopy, fluorescence microplate assays, or flow cytometry.

KEY PARAMETERS

Flow cytometer

Excitation	488 nm laser
Emission	530/30 nm filter
Instrument specification(s)	FITC channel

Fluorescence microscope

Excitation	FITC filter set
Emission	FITC filter set
Recommended plate	Black wall/clear bottom

Fluorescence microplate reader

Excitation	490
Emission	525
Cutoff	515
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.

OG488 BAPTA-1 AM Stock Solution
Prepare a 2 to 5 mM stock solution of OG488 BAPTA-1 AM in high-quality, anhydrous DMSO.

PREPARATION OF WORKING SOLUTION

OG488 BAPTA-1 AM Working Solution
On the day of the experiment, either dissolve OG488 BAPTA-1 AM in DMSO or thaw an aliquot of the indicator stock solution to room temperature. Prepare a dye working solution of 2 to 20 µM in a buffer of your choice (e.g., Hanks and Hepes buffer) with 0.04% Pluronic® F-127. For most cell lines, OG488 BAPTA-1 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 OG488 BAPTA-1 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 solution, 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 OG488 BAPTA-1 AM working solution into 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

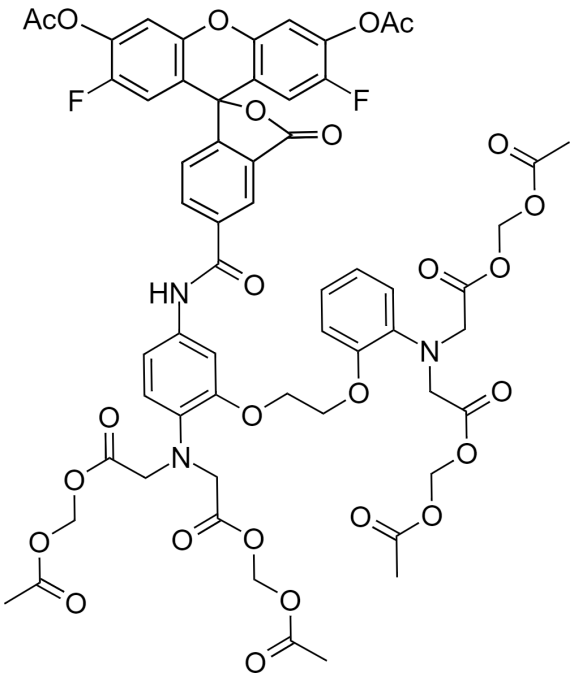

Figure 1. Chemical structure for OG488 BAPTA-1, AM [equivalent to Oregon Green® 488 BAPTA-1, AM] *Cell permeant*

DISCLAIMER

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