Fluo-8H™, AM

Catalog number: 21090, 21091
Unit size: 1 mg, 10x50 ug

Component	Storage	Amount (Cat No. 21090)	Amount (Cat No. 21091)
Fluo-8H™, AM	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)	10x50 ug

OVERVIEW

Calcium measurements are critical for numerous biological investigations. Fluorescent probes that show spectral responses upon binding Ca2+ have enabled researchers to investigate changes in intracellular free Ca2+ concentrations by using fluorescence microscopy, flow cytometry, fluorescence spectroscopy, and fluorescence microplate readers. Fluo-3 AM and Fluo-4 AM are most commonly used among the visible light-excitable calcium indicators for live-cell calcium imaging. However, Fluo-3 AM and Fluo-4 AM are only moderately fluorescent in live cells upon esterase hydrolysis and require harsh cell loading conditions to maximize their cellular calcium responses. Fluo-8® dyes are developed to improve cell loading and calcium response while maintaining the convenient Fluo-3 and Fluo-4 spectral wavelengths of Ex/Em = ~490/~520 nm. Fluo-8® AM can be loaded into cells at room temperature, while Fluo-3 AM and Fluo-4 AM require 37°C for cell loading. In addition, Fluo-8® AM is two times brighter than Fluo-4 AM and four times brighter than Fluo-3 AM. AAT Bioquest offers a set of our outstanding Fluo-8® reagents with different calcium-binding affinities (Fluo-8® Kd = 389 nM; Fluo-8H™ Kd = 232 nM; Fluo-8L™ Kd = 1.86 µM; Fluo-8FF™ Kd = 10 µM). We also offer versatile packing sizes to meet your special needs (e.g., 1 mg, 10x50 µg, 20x50 µg, and HTS packages) with no additional packaging charge.

KEY PARAMETERS

Fluorescence microscope

Excitation	FITC
Emission	FITC
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.

Fluo-8H™ AM Stock Solution
Prepare a 2 to 5 mM stock solution of Fluo-8H™ AM in high-quality, anhydrous DMSO.

PREPARATION OF WORKING SOLUTION

Fluo-8H™ AM Working Solution
On the day of the experiment, either dissolve Fluo-8H™ 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, Fluo-8H™ 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 Fluo-8H™ 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 Fluo-8H™ 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 2 hours 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 490/525 nm cutoff 515 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

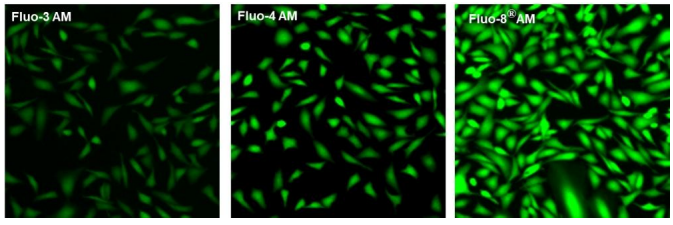

Figure 1. U2OS cells were seeded overnight at 40,000 cells per 100 uL per well in a 96-well black all/clear bottom costar plate. The growth medium was removed, and the cells were incubated with 100 uL of 4 uM Fluo-3 AM, Fluo-4 AM or Fluo-8® AM in HHBS at 37 °C for 1 hour. The cells were washed twice with 200 uL HHBS, then imaged with a fluorescence microscope using FITC channel.

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.