

## Fluo-8®, AM

 Catalog number: 21080, 21081, 21082, 21083  
 Unit size: 1 mg, 5x50 ug, 10x50 ug, 20x50 ug

Component	Storage	Amount (Cat No. 21080)	Amount (Cat No. 21081)	Amount (Cat No. 21082)	Amount (Cat No. 21083)
Fluo-8®, AM	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)	5x50 ug	10x50 ug	20x50 ug

### OVERVIEW

Calcium measurements are critical for numerous biological investigations. Fluorescent probes that show spectral responses upon binding Ca<sup>2+</sup> have enabled researchers to investigate changes in intracellular free Ca<sup>2+</sup> 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-excitability 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® K<sub>d</sub> = 389 nM; Fluo-8H™ K<sub>d</sub> = 232 nM; Fluo-8L™ K<sub>d</sub> = 1.86 μM; Fluo-8FF™ K<sub>d</sub> = 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-8® AM Stock Solution

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

### PREPARATION OF WORKING SOLUTION

#### Fluo-8® AM Working Solution

On the day of the experiment, either dissolve Fluo-8® 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-8® 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-8® 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-8® AM working solution into your cell plate.

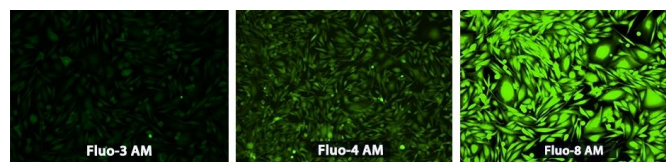
**Note** If your compound(s) interfere with the serum, replace the growth medium with fresh HHBS buffer before dye-loading.

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/100 μL/well in a 96-well black wall/clear bottom costar plate. The growth medium was removed, and the cells were incubated with, respectively, 100 μL of Fluo-3 AM, Fluo-4 AM and Fluo-8® AM in HHBS at a concentration of 4 μM in a 37 °C, 5% CO<sub>2</sub> incubator for 1 hour. The cells were washed twice with 200 μL HHBS, then imaged with a fluorescence microscope (Olympus IX71) 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

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