# Fluo-5F, AM *Cell permeant*

Catalog number: 20560
Unit size: 10x50 ug

| Component | Storage | Amount |
|---|---|---|
| Fluo-5F, AM *Cell permeant* | Freeze (< -15 °C), Minimize light exposure | 10x50 ug |

## OVERVIEW

Fluo-5F is an analog of Fluo-4 with lower calcium-binding affinity (Kd = ~2.3 uM), making it suitable for detecting intracellular calcium levels in the range of 1 µM to 1 mM that would saturate the response of Fluo-4. Cells may be loaded with Fluo-5F AM ester by adding the dissolved indicator directly to dishes containing cultured cells. It is compatible with excitation at 488 nm by argon-ion laser sources, making Fluo-5F useful for confocal microscopy, flow cytometry, and microplate screening applications. It has excitation and emission wavelengths at 494 and 516 nm respectively. Upon calcium binding, its fluorescence intensity increases by >100 fold.

## KEY PARAMETERS

**Flow cytometer**

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

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

## PREPARATION OF WORKING SOLUTION

**Fluo-5F AM Working Solution**
On the day of the experiment, either dissolve Fluo-5F 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-5F 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-5F 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-5F 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

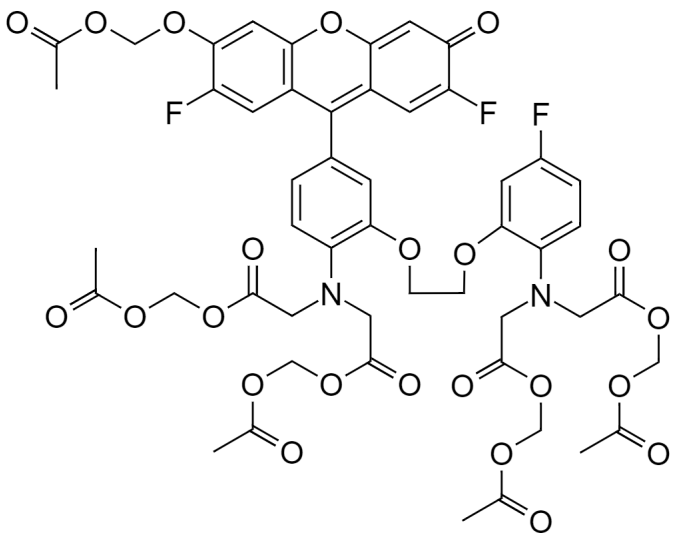

**Figure 1.** Chemical structure for Fluo-5F, AM *Cell permeant*

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