

## Fluo-4, AM

Catalog Number	Packaging Size
C219	0.5 mg
C220	10×50 µg
C221	0.5 ml

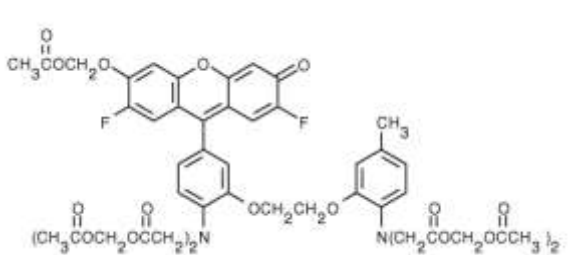
**Storage upon receipt:** -20°C, protected from light

### Introduction

**Fluo-4, AM** is an analog of Fluo-3, AM with the two chlorine substituents replaced by fluorines, which results in increased fluorescence excitation at 488 nm and consequently higher fluorescence signal levels. Fluo-4, AM is a membrane permeant probe and can be passively loaded in cells by simple incubation. Once inside the cell, Fluo-4 becomes fluorescent in the presence of free Ca<sup>2+</sup>. Fluo-4, AM is widely used for determining GPCR function via cell-based high-throughput screening. This indicator is useful for fluorescence and confocal microscopy, flow cytometry, and microplate screening applications.

**Fluo-4, AM** is supplied as 0.5 mg solid (**C219**), special packing 10×50 µg (**C220**), and 1 mg/ml solution in DMSO (**C221**).

### Specifications

<b>Label:</b>	Fluo-4	
<b>Ex/Em:</b>	488/506 nm	
<b>Detection Method:</b>	Fluorescent	
<b>Molecular Formula:</b>	C <sub>51</sub> H <sub>50</sub> F <sub>2</sub> N <sub>2</sub> O <sub>23</sub>	
<b>Molecular Weight:</b>	1096.95	
<b>CAS Number:</b>	-	
<b>Storage Conditions:</b>	-20°C, protect from light	
<b>Shipping Condition:</b>	Room Temperature	

### Applications

#### Cell Loading Guideline

Note: The following protocol is provided as an introductory guide only. The detailed procedures can be found from literatures.<sup>1,2</sup>

1. Prepare a Fluo-4 AM stock solution in anhydrous DMSO at 1-5 mM.

2. Dilute an aliquot of Fluo-4 AM stock solution (1-5 mM) to a final concentration of 1-5  $\mu$ M in the buffered physiological medium of choice. Addition of the non-ionic detergent Pluronic R F-127 can assist in dispersion of the nonpolar Fluo-4 AM ester in aqueous media. This can be conveniently accomplished by mixing the aliquot of Fluo-4 AM ester stock solution in DMSO with an equal volume of 20% (w/v) Pluronic in DMSO (Cat No. C021) before dilution into the loading medium, making the final Pluronic concentration about 0.02%.
3. The organic anion-transport inhibitors probenecid (1-2.5 mM) may be added to the cell medium to reduce leakage of the de-esterified indicator.
4. Cells are normally incubated with the Fluo-4 AM ester for 15–60minutes at 20–37°C. Exact loading concentration, time, and temperature will need to be determined empirically; in general it is desirable to use the minimum dye concentration required to yield fluorescence signals with adequate signal to noise. Subcellular compartmentalization, an inherent problem with the AM ester loading technique, is usually lessened by lowering the incubation temperature.
5. Before fluorescence measurements are commenced, cells should be washed in indicator-free medium (containing an anion transport inhibitor, if applicable) to remove any dye that is nonspecifically associated with the cell surface, and then incubated for a further 30 minutes to allow complete de-esterification of intracellular Fluo-4 AM ester.

### High-Throughput Screening

Intracellular  $\text{Ca}^{2+}$  measurements in 96-well and 384-well microplates are an essential tool for high-throughput pharmacological screening. Cell samples in microplate wells are loaded with the AM ester form of the indicator using protocol basically similar to those described in ***Cell Loading Guideline***.

### References:

1. Methods Cell Biol 40, 155 (1994);
2. *Cell Biology: A Laboratory Handbook, 2nd Edition*, J.E. Celis, Ed., Volume 3, pp 363–374, Academic Press (1998);