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Fluo-2, AM

Catalog Number	Packaging Size
C207	1 mg
C208	20×50 µg
C209	1 ml

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

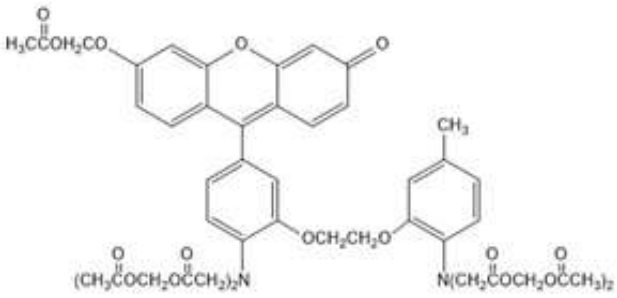
Introduction

Fluo-2, AM is one of the original Fluo series of Ca^{2+} indicators invented by Roger Tsien in the 1980s. Fluo-2, AM has been found to be much brighter (1.5x) than Fluo-4, AM in cellular experiments. The increased brightness of Fluo-2 is partly attributable to its superior loading in most cell types.

Fluo-2, AM is supplied as 1 mg solid (**C207**), special packing 20×50 µg (**C208**), and 1 mg/ml solution in DMSO (**C209**).

Specifications

Label:	Fluo-2
Ex/Em:	488/515 nm
Detection Method:	Fluorescent
Molecular Formula:	$C_{51}H_{52}N_2O_{23}$
Molecular Weight:	1061
CAS Number:	-
Storage Conditions:	-20°C, protect from light
Shipping Condition:	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.[1,2]

1. Prepare a Fluo-2 AM stock solution in anhydrous DMSO at 1-5 mM.
2. Dilute an aliquot of Fluo-2 AM stock solution (1-5 mM) to a final concentration of 1-5 µM 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-2 AM ester in aqueous media. This can be conveniently accomplished by mixing the aliquot of Fluo-2 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-2 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-2 AM ester.

High-Throughput Screening

Intracellular 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);