

## Product Information

### Calcein blue, AM and Calcein, AM

Catalog Number	Product Name	Unit Size
C022	Calcein blue, AM	1 mg
C023	Calcein blue, AM	500 µL
C025	Calcein, AM	1 mg
C026	Calcein, AM	500 µL

#### Storage upon receipt:

- -20°C
- Protect from light

### Product Description

The acetoxymethyl (AM) ester derivatives of fluorescent indicators and chelators make up one of the most useful groups of compounds for the study of live cells. Modification of carboxylic acids with AM ester groups results in an uncharged molecule that can permeate cell membranes. Once inside the cell, the lipophilic blocking groups are cleaved by nonspecific esterases, resulting in a charged form that leaks out of cells far more slowly than its parent compound. Frequently, hydrolysis of the esterified groups is essential for binding of the target ion. In some cases (e.g., calcein AM), the AM ester is colorless and nonfluorescent until hydrolyzed. This property is useful in diagnosing spontaneous hydrolysis during storage. Acetate groups, used on many fluorescent indicators, are analogous to AM ester groups and should be treated similarly.

### Guidelines for Use

#### Preparing the Stock Solutions

AM or acetate esters should be reconstituted only as required using high-quality, anhydrous dimethylsulfoxide (DMSO). Reagent-grade DMSO should be stored well sealed under argon or nitrogen, and desiccated. Dissolution of the pure AM or acetate esters in DMSO may be slow (particularly in the 1 mg sizes). Once prepared, DMSO stock solutions of AM or acetate esters should preferably be used within a short time period for one series of experiments. DMSO stock solutions must be kept anhydrous, since the solvent will readily take up moisture, leading to decomposition of the dye. These stock solutions should be stored well sealed, frozen, and desiccated.

It is advisable to keep the AM ester or acetate ester in as concentrated a stock as possible so that minimal amounts (ideally  $\leq 0.1\%$ ) of DMSO are present in the loading solution.

#### Using Pluronic® F-127 Dispersing Agent

Since some AM esters are relatively insoluble in aqueous solutions, the low-toxicity dispersing agent Pluronic® F-127 is often used to facilitate cell loading. This nonionic detergent can be made up to a final concentration of 20% (w/v) in DMSO, and this solution can be used to prepare the dye stock. Gentle warming ( $\sim 40^\circ\text{C}$ ) may assist in getting the detergent into DMSO. Pluronic® F-127 may decrease the stability of AM esters, so it should only be added to working stocks.

Whether Pluronic® is used or not, it is advisable to keep the AM ester or acetate ester in as concentrated a stock as

possible so that minimal amounts (ideally  $\leq 0.1\%$ ) of DMSO are present in the loading solution.

#### Loading of Cells with AM or Acetate Esters

This is intended as an introduction only. Specific protocols for any particular dye and cell type should be obtained from the literature. As a rule, AM and acetate esters are used at a final working concentration of between 1 and 10  $\mu\text{M}$ . Higher concentrations of weakly fluorescent indicators. The AM or acetate ester concentration should be kept as low as possible to reduce potential artifacts from overloading, including incomplete hydrolysis, compartmentalization, and toxic effects of hydrolysis byproducts such as formaldehyde or acetic acid. Generally, loading times of between 15 minutes and 1 hour are sufficient. Loading may be done at a temperature that is optimal for the cells, although some investigators have reported greater degrees of compartmentalization at physiological temperatures than at room temperature. In addition to assisting in dye uptake, Pluronic® F-127 may help in reducing compartmentalization. To keep extracellular hydrolysis of the AM and acetate esters to a minimum, it is recommended that a loading buffer free of primary and secondary amines such as PBS be used. Cells should be washed in dye-free buffer after loading.