Acetoxymethyl (AM) and Acetate Esters

Table 1. Contents and storage information.

Product	Amount	Concentration	Storage	Stability
Please refer to product label			• ≤–20°C • Desiccated • Protected from light **or** • ≤–20°C • Desiccated *(see the label)*	When stored as directed, product is stable for 6 months.

Introduction

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; desiccant beads (e.g., molecular sieves) can be used for short-term storage. 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. Many of our AM and acetate esters are available in small aliquots. Use of the prealiquoted product is strongly recommended.

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

Using Pluronic® F-127 Dispersing Agent

Since some AM esters (particularly SBFI, AM and PBFI, AM) 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 (~40°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.

For the convenience of our customers, Molecular Probes offers Pluronic® F-127 in three forms: 1 mL of a 20% (w/v) solution in DMSO (P3000), 30 mL of a 0.2 µm–filtered 10% (w/v) solution in water (P6866) and 2 g solid (P6867).

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 ≤ 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 µM. Higher concentrations of weakly fluorescent indicators such as Fura Red™ and quin-2 may be required. 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, although probes such as SBFI, AM and PBFI, AM may require 1–4 hours. 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.

Assessing Dye Responsiveness

The following protocol is optional and can be used to test the responsiveness of the dye (for example following extended storage) or as a means to calibrate the ion response of the dye. Following the calibration or testing of the dye using this protocol, it should be disposed of and not loaded into cells.

We generally recommend that the separately available salt or free acid form of an indicator be used for calibrating the ion response. However, the following protocol for AM or acetate ester hydrolysis provides a less-preferred alternative and may also be useful to assess spontaneous hydrolysis during storage. *This procedure is not always successful for AM esters, probably because of the formation of formaldehyde in the reaction.*

1.1 Dissolve a small amount of the AM or acetate ester (e.g., 50 µg calcein AM) in 50 µL dioxane, DMSO, or other water-miscible solvent.

1.2 Add an equal volume of methanol.

1.3 Add 25 µL of 2 M KOH/water. If the dye is not in solution at this point, add more methanol.

1.4 Wait one hour.

1.5 Adjust pH to ~7 with HCl.

1.6 Test for fluorescent response. For example, to test a calcium indicator, dilute 5 µL of the dye solution into 100 µL of water, add this separately to high-calcium buffer and to low-calcium buffer.

1.7 If the dye does not respond properly, add more KOH/methanol to the dye solution and repeat steps 1.6 and 1.7.

Related Products

Current prices may be obtained from our website or from our Customer Service Department.

Cat #	Product Name	Unit Size
P6867	Pluronic® F-127 *low UV absorbance*	2 g
P3000	Pluronic® F-127 *20% solution in DMSO*	1 mL
P6866	Pluronic® F-127 *10% solution in water* *0.2 µm filtered*	30 mL
P10020	PowerLoad™ 100X concentrate	5 mL
P36400	Probenecid, water soluble	10 × 77 mg

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