

Acetoxymethyl (AM) and Acetate Esters

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.

Storage and Handling

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 pre-aliquoted product is strongly recommended.

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. 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. 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 (P-3000), 30 mL of a 0.2 µm–filtered 10% (w/v) solution in water (P-6866) and 2 g solid (P-6867).

Loading of Cells with AM or Acetate Esters

The following sample protocol 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 by-products 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.

Chemical Hydrolysis of AM Esters and Diacetates

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.

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
P-6867	Pluronic® F-127 *low UV absorbance*	2 g
P-3000	Pluronic® F-127 *20% solution in DMSO*	1 mL
P-6866	Pluronic® F-127 *10% solution in water* *0.2 µm filtered*	30 mL

Contact Information

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

Please visit our Web site — www.probes.com — for the most up-to-date information

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