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Preparation of Dihydrorhod-2 AM

Introduction

The cell-permeant AM ester derivative of rhod-2 (R-1244, R-1245) has a net positive charge. This property promotes its sequestration into mitochondria in some cells, most likely via membrane potential–driven uptake. By reducing rhod-2 AM to the colorless, nonfluorescent dihydrorhod-2 AM, the discrimination between cytosolic and mitochondrially localized dye can be further improved.[1] The AM ester of dihydrorhod-2 exhibits Ca^{2+}-dependent fluorescence only after it is oxidized and its AM esters are cleaved to yield the rhod-2 indicator, processes that occur rapidly in the mitochondrial environment. Reduction of rhod-2 AM (R-1244, R-1245) to dihydrorhod-2 AM can be readily accomplished using the protocol given below.

Protocol

1.1 Dissolve 50 µg of rhod-2 AM (one vial of the set of 20 supplied as product R-1245) in 100 µL of anhydrous dimethylsulfoxide (DMSO).

1.2 Add a small excess of sodium borohydride ($NaBH_4$) either as a solid or as a methanol solution. The smallest amount of solid $NaBH_4$ that can be practicably transferred will provide a sufficient excess.

1.3 Incubate for 10 minutes or until the reaction mixture appears colorless, whichever is sooner.

1.4 Use the reaction solution in DMSO (about 0.4 mM dihydrorhod-2 AM) directly for cell loading according to usual AM ester loading protocols (see our product information sheets *Rhod-2 and X-rhod-1 Calcium Indicators* (MP1244) or *Acetoxymethyl (AM) Esters* (G002)).

1.5 Dihydrorhod-2 AM will spontaneously and quite rapidly revert to the oxidized form, marked by reappearance of color in the stock solution. Therefore experiments using dihydro rhod-2 AM should be carried out as soon as possible after preparation.

References

1. Cell 82, 415 (1995).

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