Multi-Color Labeling and Functional Analysis of Live Cells Using Fluorescent Calcein AM Dyes

Introduction

Calcein AM is a hydrophobic compound that easily permeates intact live cells. It is widely used in cell viability assays. The hydrolysis of the non-fluorescent Calcein AM by intracellular esterases generates the strongly fluorescent hydrophilic Calcein that is well-retained in the cell cytoplasm. The esterase activity is proportional to the number of vial cells, and thus directly related to the fluorescence intensity of Calcein generated from the esterase-catalyzed hydrolysis of Calcein AM. Calcein AM is one of the most popular fluorescent probes used for labeling and monitoring cellular functions of live cells. However, the single color of Calcein AM makes it impossible to use this valuable reagent in the multicolor applications. For example, it is impossible to use Calcein AM in combination with GFP-transfected cells due to the fact that Calcein AM has the same color as GFP.

Spectral Properties of Calcein Dyes

To address the above-mentioned color limitation of Calcein AM, we have developed Calcein Orange™ and Calcein Red™. These two new Calcein AM analogs enable the multicolor labeling and functional analysis of live cells in combination with Calcein AM. In addition, we have also developed CytoCalcein™ Violet 450, CytoCalcein™ Violet 500, CytoCalcein™ Blue 550 and CytoCalcein™ Blue 600 for flow cytometric applications. CytoCalcein™ dyes exhibit similar biological properties to Calcein, AM. They are optimized for the excitation wavelengths of a variety of flow cytometers, providing additional colors for flow cytometric analysis of live cells. CytoCalcein™ Violet 450 and CytoCalcein™ Violet 500 are well excited by violet laser (405 nm) and emit fluorescence at 450 nm and 500 nm respectively. CytoCalcein™ Blue 550 and CytoCalcein™ Blue 550 are well excited by blue laser (488 nm) and emit fluorescence at 550 nm and 600 nm respectively.

Table 1. Spectral Properties of Cell Viability Detection Reagents

Indicators	Product Number	Excitation	Emission
Calcein, AM	22002, 22003 and 22004	490 nm	515 nm
Calcein Blue, AM	22007	360 nm	445 nm
Calcein Orange™	22009	525 nm	550 nm
Calcein Red™	22010	646 nm	659 nm
CytoCalcein™ Violet 450	22012	408 nm	450 nm
CytoCalcein™ Violet 500	22013	408 nm	500 nm

Use of Cell Viability Indicator AM Esters

AM esters are the non-polar esters that readily cross live cell membranes, and rapidly hydrolyzed by cellular esterases inside live cells. AM esters are widely used for loading a variety of polar fluorescent probes into live cells non-invasively. However, cautions must be excised when AM esters are used since they are susceptible to hydrolysis, particularly in solution. They should be reconstituted just before use in high-quality, anhydrous dimethylsulfoxide (DMSO). DMSO stock solutions may be stored desiccated at -20 °C and protected from light. Under these conditions, AM esters should be stable for several months.

Following is our recommended protocol for loading AM esters into live cells. This protocol only provides a guideline, and should be modified according to your specific needs.

a) Prepare a 2 to 5 mM stock solution of AM esters in high-quality, anhydrous DMSO. The nonionic detergent Pluronic® F-127 is sometimes used to increase the aqueous solubility of AM esters. *Note: An equal volume of 20% Pluronic ® F-127 solution can be added to DMSO stock solutions of cell viability indicator before diluting into the loading buffer. The final Pluronic ®F-127*

concentration is about 0.02%. A variety of Pluronic® F-127 solutions can be purchased from AAT Bioquest.
Caution: The long-term storage of AM esters in the presence of Pluronic® F-127 is not recommended.

b) On the day of the experiment, either dissolve the solid indicators of cell viability in DMSO or thaw an aliquot of the indicator stock solutions to room temperature. Prepare a working solution of 1 to 10 µM in the buffer of your choice (such as Hanks and Hepes buffer). For most of the cell lines, cell viability indicators at the final concentration ranging from 4 to 5 µM are recommended. The exact concentration of indicators required for cell loading must be determined empirically.
c) If your cells containing the organic anion-transports, probenecid (1–2.5 mM) or sulfinpyrazone (0.1–0.25 mM) may be added to the cell medium to reduce leakage of the de-esterified indicators. Incubate cells with AM esters at room temperature or 37 °C for 20 minutes to one hour.
Note: Decreasing the loading temperature might reduce the indicator compartmentalization.
d) Wash cells in cell viability indicator-free buffer (containing an anion transporter inhibitor, if applicable) to remove excess probes.
Note: Cell samples were immediately analyzed by flow cytometry to determine the average fluorescence per cell at time zero without washing.
e) Run the experiments at the desired Ex/Em wavelengths (see Table 1).

Storage Conditions

Store at –20 ^{0}C, protected from light. Expiration date is 6 months from the date of receipt.

References

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