Calcein Deep Red™ AM ester

Catalog number: 22011
Unit size: 1 mg

Component	Storage	Amount
Calcein Deep Red™ AM ester	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)

OVERVIEW

Calcein AM is one 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 of GFP-tranfacted cells due to the same color to GFP. To address this color limitation of Calcein AM, we have developed Calcein Orange™, Calcein Red™ and Calcein Deep Red™. These new Calcein AM ester enables the multicolor labeling and functional analysis of live cells in combination with Calcein AM. Non-fluorescent Calcein Deep Red™ AM ester can easily get into live cells and hydrolyzes to generate strongly fluorescent Calcein Deep Red™ (Cat#: 21902) dye. Calcein Deep Red™ dye can be monitored with the common Cy5 filter set.

KEY PARAMETERS

Flow cytometer

Excitation	633/640 nm laser
Emission	660/20 nm filter
Instrument specification(s)	APC channel

Fluorescence microscope

Excitation	Cy5 filter set
Emission	Cy5 filter set
Recommended plate	Black wall/clear bottom

Fluorescence microplate reader

Excitation	620
Emission	660
Cutoff	630
Recommended plate	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

Calcein Deep Red™ AM ester stock solution
Prepare a 2 to 5 mM stock solution of Calcein Deep Red™ AM in high-quality, anhydrous DMSO.

Note The nonionic detergent Pluronic® F-127 can be used to increase the aqueous solubility of AM esters. In the staining buffer, the final Pluronic® F-127 concentration should be approximately 0.02%. A variety of Pluronic® F-127 products can be purchased from AAT Bioquest. Avoid long-term storage of AM esters in the presence of Pluronic® F-127.

PREPARATION OF WORKING SOLUTION

Calcein Deep Red™ AM ester working solution
Prepare a Calcein Deep Red™ AM working solution of 1 to 10 µM in the buffer of your choice (e.g., Hanks and Hepes buffer). For most cell lines, Calcein Deep Red™ AM at the final concentration of 4 to 5 µM is recommended. The exact concentration of indicators required for cell loading must be determined empirically.

Note If your cells contain organic anion-transporters, probenecid (1–2.5 mM) or sulfinpyrazone (0.1–0.25 mM) may be added to the working solution to reduce leakage of the de-esterified indicators.

SAMPLE EXPERIMENTAL PROTOCOL

1. Prepare cells for imaging.
2. Remove the cell culture medium and wash cells once with serum-free buffer to remove any remaining media.

   Note Serum in cell culture media may contain esterase activity, which can increase background interference.
3. Add Calcein Deep Red™ AM working solution to the culture.
4. Incubate cells at 37 °C for 30 to 60 minutes.
5. Replace the dye working solution with HHBS or buffer of your choice (containing an anion transporter inhibitor, such as 1 mM probenecid, if applicable) to remove any excess probes.
6. Measure the fluorescence intensity using either a fluorescence microscope equipped with a Cy5 filter set, a flow cytometer equipped with a 660/20 nm filter (APC channel), or a fluorescence plate reader at Ex/Em = 620/660 nm cutoff 630 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

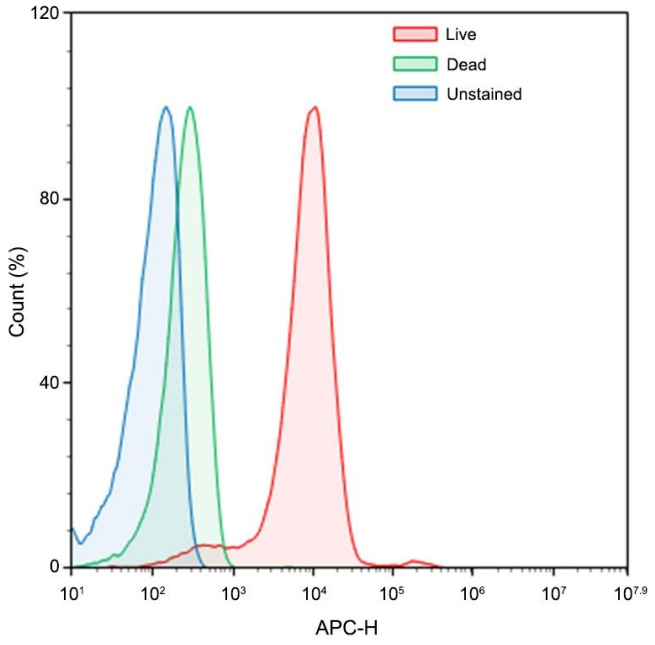

Figure 1. Flow Cytometry Analysis of Jurkat cells stained with Calcein Deep Red™ AM ester (Cat#22011). Jurkat cells were washed once with HH buffer and stained with 2 uM Calcein Deep Red™ AM ester (Cat#22011) in HH with 0.02% PF-127(Cat#20053) and 1mM PBC (Cat# 20061) for 30 minutes at 37C incubator. Cells were then washed with HH buffer and resuspended in HH buffer. The fluorescence intensities of Live cells (healthy, Red) and Dead cells (treated

in 55°C water bath for 30 minutes, Green) were measured with NovoCyte 3000 flow cytometer using blue laser APC emission channel.

DISCLAIMER

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