Calcein UltraGreen™ AM

Catalog number: 21905
Unit size: 10x50 ug

Component	Storage	Amount (Cat No. 21905)
Calcein UltraGreen™ AM	Freeze (< -15 °C), Minimize light exposure	10x50 ug

OVERVIEW

Calcein UltraGreen™ AM readily passes through the cell membrane of viable cells. Upon transporting into live cells cellular esterases cut off the AM groups, the molecule gets trapped inside cells. Compared with Calcein AM, Calcein UltraGreen™ is more suitable fluorescent probe for staining viable cells because of its lower cytotoxicity and longer retention in cells. UltraGreen™ AM does not significantly affect cellular functions such as proliferation or chemotaxis of lymophocyte.

KEY PARAMETERS

Flow cytometer

Emission	530/30 nm filter
Excitation	488 nm laser
Instrument specification(s)	FITC channel

Fluorescence microscope

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

Fluorescence microplate reader

Cutoff	515
Emission	525
Excitation	490
Recommended plate	Black wall/clear bottom
Instrument specification(s)	Bottom read mode

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 UltraGreen™ AM Stock Solution

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

   Note: When reconstituted in DMSO, Calcein UltraGreen™ AM is a clear, colorless solution.

PREPARATION OF WORKING SOLUTION

Calcein UltraGreen™ AM Working Solution

1. Prepare a Calcein UltraGreen™ 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 UltraGreen™ 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: 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.

   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. A variety of ReadiUse™ Probenecid products, Including water-soluble, sodium salt, and stabilized solutions, can be purchased from AAT Bioquest.

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 UltraGreen™ 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 FITC filter set, a flow cytometer equipped with a blue laser and a 530/30 nm filter (FITC channel), or a fluorescence plate reader at Ex/Em = 490/525 nm cutoff 515 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

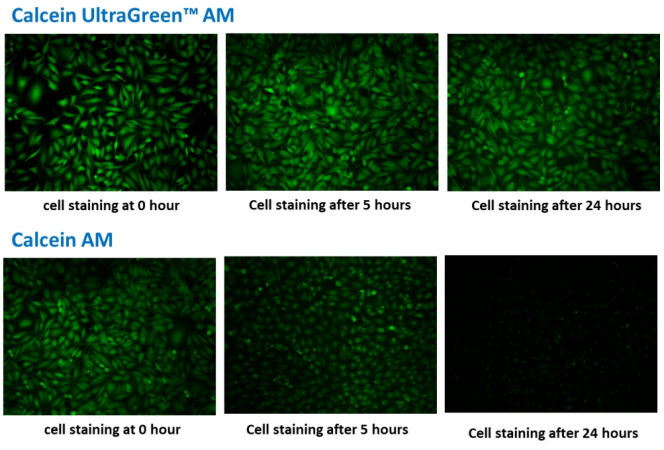

Figure 1. Fluorescence images of HeLa cells stained with Calcein UltraGreen™ AM (upper row) or Calcein AM (lower row) in a Costar black wall/clear bottom 96-well plate. After washing, growth media were added back, and the cells were monitored using a microscope with FITC filter for up to 24 hours.

DISCLAIMER

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