Calcein, AM *UltraPure grade* *CAS 890090-35-4*

Catalog number: 22003, 22004
Unit size: 1 mg, 20x50 ug

Component	Storage	Amount (Cat No. 22003)	Amount (Cat No. 22004)
Calcein, AM *UltraPure grade* *CAS 890090-35-4*	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)	20x50 ug

OVERVIEW

Calcein-AM is a green fluorogenic dye that stains live cells for cell viability in flow cytometry and fluorescence microscopy. It is the acetoxymethyl (AM) ester form of the fluorescent probe, calcein.

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, AM *UltraPure grade* stock solution

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

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

PREPARATION OF WORKING SOLUTION

Calcein, AM *UltraPure grade* working solution

1. Prepare a Calcein 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 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 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

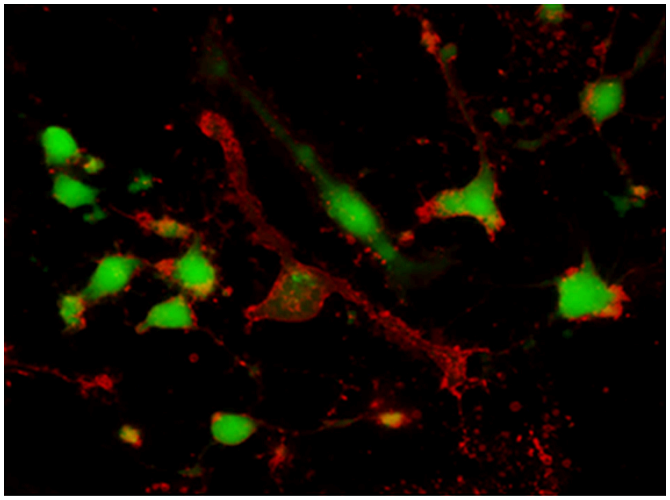

Figure 1. Simultaneous imaging of live and apoptotic HeLa cells labeled using calcein AM (Cat No. 22003) and Annexin V-iFluor® 647 conjugate (Cat No. 20074)

DISCLAIMER

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