Calcein Blue, AM *CAS 168482-84-6*

Catalog number: 22007
Unit size: 1 mg

Component	Storage	Amount
Calcein Blue, AM *CAS 168482-84-6*	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)

OVERVIEW

It is a cell-permeable version of calcein blue. Upon getting into live cells the weakly fluorescent calcein blue AM is hydrolyzed into calcein blue that has the excitation/emission maxima similar to those of DAPI, Hoechst and AMCA. This exceptional spectral separation from the typical green and red fluorophores (such as FITC, TMR and Texas Red) provides additional options for multiplexing experiments. Because calcein blue AM is intrinsically fluorescent, a proper filter set and additional wash step may be necessary to minimize background fluorescence. Our calcium assay buffers may be used to effectively wash extracellular calcein blue.

KEY PARAMETERS

Flow cytometer

Excitation	350 nm or 405 nm laser
Emission	450/40 nm filter
Instrument specification(s)	Pacific Blue channel

Fluorescence microscope

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

Fluorescence microplate reader

Excitation	360
Emission	450
Cutoff	420
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 Blue, AM Stock Solution
Prepare a 2 to 5 mM stock solution of Calcein Blue 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 Blue, AM Working Solution
Prepare a Calcein Blue 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 Blue 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 Blue 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 DAPI filter set, a flow cytometer equipped with a 450/40 nm filter (Pacific Blue channel), or a fluorescence plate reader at Ex/Em = 360/450 nm cutoff 420 nm.

DISCLAIMER

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