# Calcein UltraBlue™ AM

Catalog number: 21908
Unit size: 10x50 ug

| Component | Storage | Amount |
|---|---|---|
| Calcein UltraBlue™ AM | Freeze (< -15 °C), Minimize light exposure | 10x50 ug |

## OVERVIEW

Calcein UltraBlue™ AM is a cell-permeable version of Calcein UltraBlue™. Upon getting into live cells the weakly fluorescent Calcein UltraBlue™ AM is hydrolyzed into Calcein UltraBlue™ that has the excitation/emission maxima similar to those of Calcein Blue, 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. Calcein UltraBlue™ has similar spectral properties to those of Calcein Blue. However, compared with Calcein Blue, Calcein UltraBlue™ has higher photostability and stronger fluorescence intensity at physiological pH, making it a more robust fluorescent probe than Calcein Blue.

## KEY PARAMETERS

**Flow cytometer**

| | |
|---|---|
| Excitation | 350/405 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 UltraBlue™ AM Stock Solution**
Prepare a 2 to 5 mM stock solution of Calcein UltraBlue™ 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 UltraBlue™ AM Working Solution**
Prepare a Calcein UltraBlue™ 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 UltraBlue™ 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 UltraBlue™ 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 UV/violet laser and a 450/40 nm filter (Pacific Blue channel), or a fluorescence plate reader at Ex/Em = 360/450 nm cutoff 420 nm.

## EXAMPLE DATA ANALYSIS AND FIGURES

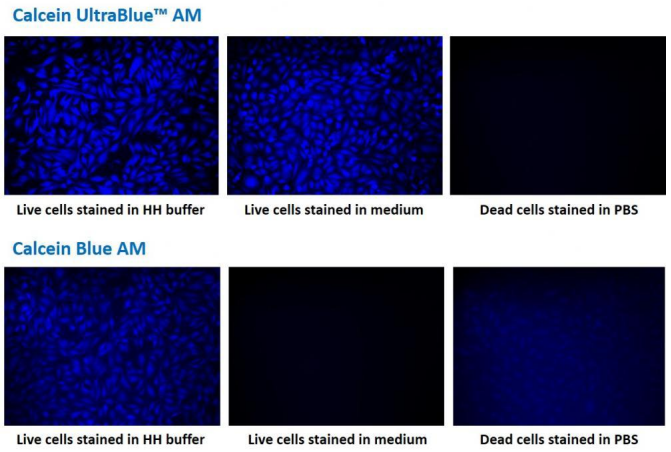


**Figure 1.** Fluorescence images of HeLa cells stained with Calcein UltraBlue™ AM (upper row) or Calcein Blue AM (lower row) in a Costar black wall/clear bottom 96-well plate. Left: Live HeLa cells in HH buffer; Middle: Live HeLa cells in medium; Right: Fixed HeLa cells.

## DISCLAIMER

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