# Calcein, AM *CAS 890090-35-4*

Catalog number: 22002
Unit size: 1 mg

| Component | Storage | Amount (Cat No. 22002) |
|---|---|---|
| Calcein, AM *CAS 890090-35-4* | Freeze (< -15 °C), Minimize light exposure | 1 vial (1 mg) |

## OVERVIEW

Calcein AM readily passes through the cell membrane of viable cells because of its enhanced hydrophobicity as compared to calcein. The acetomethoxy (AM) derivate of calcein (calcein AM) is widely used for labeling live cells as it can be transported through the cellular membrane into live cells. The AM ester groups mask the part of the molecule that chelates calcium. Upon transporting into live cells cellular esterases cut off the AM groups, the molecule binds to calcium within cell (resulting in acquiring strong green fluorescence), and gets trapped inside. As dead cells lack esterases, only live cells are marked. This feature makes it very useful for testing of cell viability and for short-term marking of cells. Compared with other live cell-labeling reagents (such as BCECF-AM and carboxy-fluorescein diacetate), calcein-AM is the most suitable fluorescent probe for staining viable cells because of its low cytotoxicity. Calcein does not significantly affect cellular functions such as proliferation or chemotaxis of lymphocyte. In addition, viability assays using calcein are reliable and correlate well with the standard 51Cr-release assay.

## 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 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 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.

Tel: 408-733-1055 | Fax: 408-733-1304 | Email: support@aatbio.com
© 2019 AAT Bioquest, Inc. Last revised April 2026. For more information and tools, please visit https://www.aatbio.com

## EXAMPLE DATA ANALYSIS AND FIGURES

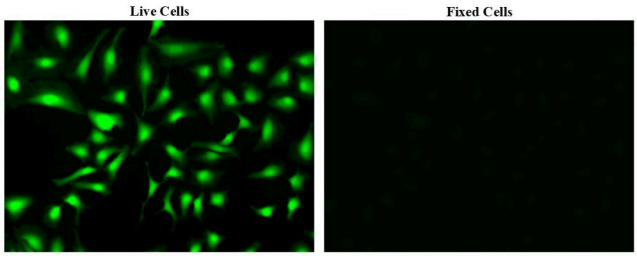


**Figure 1.** Images of live and fixed HeLa cells stained with Calcein, AM (Cat.22002) showing specificity for live cells.

## DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.


© 2019 AAT Bioquest, Inc. Last revised April 2026. For more information and tools, please visit https://www.aatbio.com