

## Calcein Red™ AM

 Catalog number: 21900  
 Unit size: 1 mg

Component	Storage	Amount
Calcein Red™ AM	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)

### OVERVIEW

Calcein AM is one of the most popular fluorescent probes used for labeling and monitoring cellular functions of live cells. However, the single color of Calcein AM makes it impossible to use this valuable reagent in the multicolor applications. For example, it is impossible to use Calcein AM in combination of GFP-transfected cells due to the same color to GFP. To address this color limitation of Calcein AM, we have developed Calcein Orange™, Calcein Red™ and Calcein Deep Red™. These new Calcein AM analogs enable the multicolor labeling and functional analysis of live cells in combination with Calcein AM. Non-fluorescent Calcein Red™ AM can easily get into live cells and hydrolyzes to generate strongly fluorescent Calcein Red™ dye. Calcein Red™ dye can be monitored with the common TRITC/Cy3 filter set. AAT Bioquest offers Calcein Red™ as a reference dye to Calcein Red™ AM.

### KEY PARAMETERS

#### Flow cytometer

Excitation 532/561 nm laser  
 Emission 585/40 nm filter

#### Fluorescence microscope

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

#### Fluorescence microplate reader

Excitation 540  
 Emission 590  
 Cutoff 570  
 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 Red™ AM Stock Solution

Prepare a 2 to 5 mM stock solution of Calcein Red™ 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 Red™ AM Working Solution

Prepare a Calcein Red™ 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 Red™ 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)

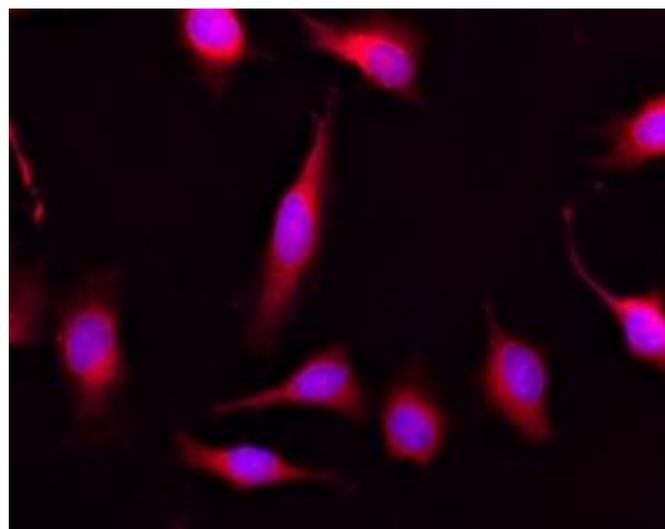
or sulfapyrazone (0.1–0.25 mM) may be added to the working solution to reduce leakage of the de-esterified indicators.

### 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 Red™ 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 TRITC filter set, a flow cytometer equipped with green/yellow laser and a 585/40 nm filter, or a fluorescence plate reader at Ex/Em = 540/590 nm cutoff 570 nm.

### EXAMPLE DATA ANALYSIS AND FIGURES



**Figure 1.** Images of Live HeLa cells stained with Calcein Red™ AM (Cat.21900). Cell nuclei were stained with Hoechst 33342 (Blue, Cat#17535).

### 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](mailto:info@aatbio.com) if you have any questions.