

## Calcein Orange™ diacetate

 Catalog number: 22009  
 Unit size: 1 mg

Component	Storage	Amount
Calcein Orange™ diacetate	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 Red™. These new Calcein AM analogs enable the multicolor labeling and functional analysis of live cells in combination with Calcein AM. Non-fluorescent Calcein Orange™ diacetate can easily get into live cells and hydrolyzes to generate strongly fluorescent Calcein Orange™ dye. AAT Bioquest also offers Calcein Orange™ as a reference dye to Calcein Orange™ diacetate.

### KEY PARAMETERS

#### Flow cytometer

Excitation	488/532 nm laser
Emission	575/26 nm filter
Instrument specification(s)	PE channel

#### Fluorescence microscope

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

#### Fluorescence microplate reader

Excitation	520
Emission	550
Cutoff	530
Recommended plate	Solid black

### 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 Orange™ Diacetate Stock Solution

Prepare a 2 to 5 mM stock solution of Calcein Orange™ diacetate 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 Orange™ Diacetate Working Solution

Prepare a Calcein Orange™ diacetate working solution of 1 to 10 μM in the buffer of your choice (e.g., Hanks and Hepes buffer). For most cell lines, Calcein Orange™ diacetate 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)

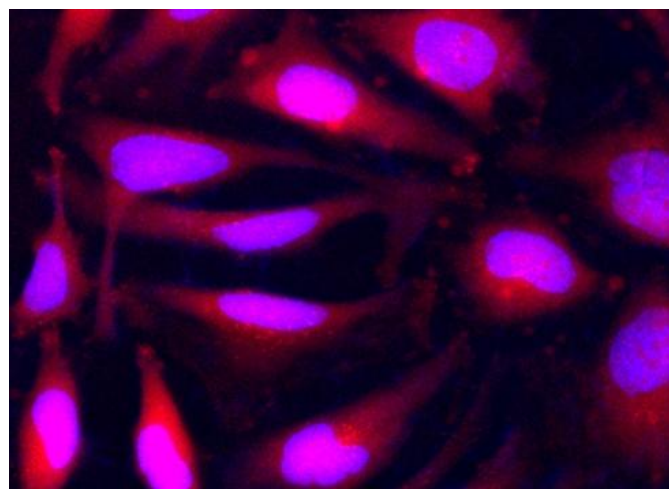
or sulfapyrazone (0.1–0.25 mM) may be added to the working solution to reduce leakage of the de-esterified indicators.

### 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 Orange™ diacetate 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 a 575/26 nm filter (PE channel), or a fluorescence plate reader at Ex/Em = 520/550 nm cutoff 530 nm.

### EXAMPLE DATA ANALYSIS AND FIGURES



**Figure 1.** Image of Live HeLa cells stained with Calcein Orange™ diacetate (Cat#22009). Cell nuclei were stained with Hoechst 33342 (Blue, Cat#17535).

### DISCLAIMER

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