Calcein Deep Red™ Acetate

Ordering Information	*Storage Conditions*
Product Number: 22010 (1 mg)	Keep at -20 °C and desiccated Expiration date is 12 months from the date of receipt

Introduction

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. In particular, the complete spectral overlap of Calcein with GFP makes it impossible to use Calcein AM for the multicolor analysis of GFP-transfected cells. To address this color limitation of Calcein AM, we have developed Calcein Orange™ and Calcein Deep Red™. These two new Calcein AM analogs enable the multicolor labeling and functional analysis of live cells in combination with Calcein AM. In addition, we have also developed CytoCalcein™ Violet 450, CytoCalcein™ Violet 500, CytoCalcein™ Blue 550 and CytoCalcein™ Blue 600 for flow cytometric applications. CytoCalcein™ dyes exhibit similar biological properties to Calcein AM. They are optimized for the excitation wavelengths of a variety of flow cytometers, providing additional colors for flow cytometric analysis of live cells. CytoCalcein™ Violet 450 and CytoCalcein™ Violet 500 are well excited by violet laser (405 nm) and emit fluorescence at 450 nm and 500 nm respectively. CytoCalcein™ Blue 550 and CytoCalcein™ Blue 550 are well excited by blue laser (488 nm) and emit fluorescence at 550 nm and 600 nm respectively.

Chemical and Physical Properties

Molecular Weight: ~500
Solvent: dimethylsulfoxide (DMSO)
Spectral Properties: Ex/Em = 647/ 659 nm

Assay Protocol with Calcein Deep Red™

Brief Summary

Prepare cells with test compounds →Remove the medium→Add Calcein Deep Red™ working solution (100 µL for a 96-well plate or 25 µL for a 384-well plate) → Incubate at room temperature or 37 °C for 1 hr → Read fluorescence intensity at Ex/Em = 635/670 nm

Note: Following is our recommended protocol for Calcein Deep Red™ live cell assay in a 96-well microplate. This protocol only provides a guideline, and should be modified according to your specific needs.

1. Prepare cells:

Plate 100 to 10,000 cells per well in a 96-well black wall/clear bottom microplate. Add test compounds into the cells for a desired period of time (such as 24, 48 or 96 hours) in a 37 °C, 5% CO_2 incubator. For blank wells (medium without the cells), add the corresponding amount of compound buffer. The total suggested volume is 100 µL for a 96-well plate, and 25 µL for a 384-well plate.
Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density for proliferation or cytotoxicity induction. For proliferation, use fewer cells, and for toxicity assays, use more cells to start with.

2. Prepare Calcein Deep Red™ working solution (for 1 plate):

2.1 Prepare a 2 to 5 mM stock solution of Calcein Deep Red™ in high-quality, anhydrous DMSO. The stock solution should be used promptly; any remaining solution should be aliquoted and refrozen at ≤ -20 °C.

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Note: Avoid repeated freeze-thaw cycles, and protect from light.

2.2 Prepare Calcein Deep Red™ working solution: On the day of the experiment, either dissolve Calcein Deep Red™ solid in DMSO or thaw an aliquot of the Calcein Deep Red™ stock solution to room temperature. Prepare a 5 to 10 µM Calcein Deep Red™ working solution in Hanks and 20 mM Hepes buffer (HHBS) or buffer of your choice pH 7, with 2.5 mM probenicid.
Note1: 10 mL of Calcein Deep Red™ working solution is not stable at room temperature; prepare fresh before use.

Note2: Many cells contain organic-anion transporters. It is highly recommended to add additional 2.5 mM probenecid, an inhibitor of organic-anion transporters, in the Calcein Deep Red™ working solution.

3. Run the cell viability assay:

3.1 Treat cells with test compounds as desired (from Step 1).
Note: It is not necessary to wash cells before compound addition. However, if tested compounds are serum sensitive, growth medium and serum factors can be aspirated away before adding compounds, provided residual volumes after the aspirate step. Alternatively, cells can be grown in serum-free media.

3.2 Remove the medium from the cells.
Note: Medium must be removed before dye loading.

3.3 Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of Calcein Deep Red™ working solution (from Step 2.2).

3.4 Incubate the Calcein Deep Red™ dye-loading plate at room temperature or 37 °C for 1 hr, protected from light.
Note 1: The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.
Note 2: For non-adherent cells, it is recommended to centrifuge cell plates at 800 rpm for 2 minutes with brake off after the incubation of the dye.

3.5 Monitor the fluorescence change at Ex/Em = 635/670 nm.
Note: If the background is high, replace the Calcein Deep Red™ working solution (from Step 3.4) with HHBS containing 2.5 mM probenicid before monitoring the fluorescence signal.

Disclaimer: *This product is for research use only and is not intended for therapeutic or diagnostic application. Please contact our technical service representative for more information.*

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