Calcein AM Dye

(Cat. # 786-1386)
INTRODUCTION
Calcein AM or Calcein acetoxymethyl ester is a hydrophobic compound, which passes easily through cell membranes into live cells and is used for cell viability assays. The non-fluorescent calcein AM dye is hydrolyzed by cellular esterases to give calcein, which is fluorescent and is retained in the cytoplasm. The intensity of calcein dye measured on a fluorimeter is directly proportional to the activity of cellular esterases, which in turn is proportional to viable cells. Calcein AM Dye is more robust than tetrazolium salts or AlamarBlue® Dye, as the cells can be stained and quantified in less than 2 hrs.

Calcein AM Dye based assays can be easily adapted to various fluorescence setups, such as microplate assays, fluorescence microscope and flow cytometry. The assay is useful for various studies, such as cell viability, cell adhesion, chemotaxis, multidrug resistance, apoptosis and cytotoxicity. The assay can be used for both suspension and adherent cells.

ITEM(S) SUPPLIED

<table>
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<tr>
<th>Cat. #</th>
<th>Description</th>
<th>Size</th>
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<tr>
<td>786-1386</td>
<td>Calcein AM Dye</td>
<td>1 mg</td>
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STORAGE CONDITIONS
Calcein AM Dye is supplied at ambient temperature. Upon arrival store at -20°C. The product when stored as directed is stable for 1 year.

IMPORTANT INFORMATION

- Protect Calcein AM dye stock solutions and working solutions protected from light as Calcien AM dye is light sensitive.
- The nonionoic detergent Pluronic® F-127 may be used to increase solubility of Calcien AM dye. An equal volume of 20 % Pluronic® F-127 can be added to Calcien AM dye DMSO stock. Final concentration of Pluronic® F-127 used in aqueous solution for cells is around 0.02%. G-Biosciences offer two formulations of Pluronic® F-127 (Cat. #786-1536, 786-1537).

**NOTE:** The long term storage of Calcien AM with Pluronic® F-127 is not recommended. So mix only the required amount of Calcien AM stock solution with equal volume of 20 % Pluronic® F-127 (Pluronic is a registered trademark of BASF).

- If the cells under study contain organic anion-transporters then probenecid (1-2.5 mM) or sulfinpyrozone (0.1-0.25 mM) is added to cell medium to reduce the leakage of calcein from cell.
PREPARATION BEFORE USE
1. Prepare 1mM stock solution of Calcein AM Dye by adding 1 ml DMSO to the Calcein AM Dye vial and mix well. Use immediately or store the solution into one time use aliquots at -20°C.
2. Prepare the working stock solution of Calcein AM Dye (1-10μM) in 1 x Hank’s balanced salt solution and 20 mM HEPES (HHBS) or buffer of choice.
   NOTE: The optimal concentration varies for different cells and should be determined by end user. The standard 2 μM Calcein AM Dye solution is suitable for NIH3T3, PtK2, HeLa and MDCK.
   NOTE: Calcein AM dye is susceptible to hydrolysis and so the working solution should be used within 2-4 hrs after preparation.

PROTOCOL

Cell Viability Assay for suspension cells
1. Plate cells in 96-well black walled cell culture plates in duplicate set. Include wells with no cells for background control.
   NOTE: The optimal seeding density should be determined by end user by plotting titration cell density curve for linear range and assay suitability for a cell type.
2. Treat the cells with or without the test compound. Perform each assay in at least duplicate set.
3. Centrifuge the microplate at 500 g for 5 minutes with centrifuge equipped to handle microplates. Alternatively, transfer cells to microfuge tubes for centrifugation and returned to plate for reading.
4. Aspirate medium from wells and wash cells once with 1 x Hank’s balanced salt solution and 20 mM HEPES (HHBS) or buffer of choice.
5. Centrifuge the microplate at 500 g for 5 minutes.
6. Add 100 μl of working stock solution of Calcein AM dye and incubate the cells for 30 minutes or 1hr in incubator (5%CO₂, 37°C).
   NOTE: For most cell types, 30 minutes incubation is adequate.
7. Measure the fluorescence on fluorescence plate reader at excitation wavelength set at 485 nm and emission wavelength at 530 nm.

Cell Viability Assay for adherent cells
1. Plate cells in 96-well black walled cell culture plates in duplicate set. Leave the cells overnight in incubator (37°C, 5%CO₂) to adhere.
   NOTE: The optimal seeding density should be determined by end user by plotting titration cell density curve for linear range and assay suitability for a cell type.
2. Next day, treat the cells with or without the test compound. Perform each assay in at least duplicate set.
3. Aspirate medium from wells and wash cells once with 1 x Hank’s balanced salt solution and 20 mM HEPES (HHBS) or buffer of choice.
4. Add 100 μl of working stock solution of Calcein AM dye and incubate the cells for 30 minutes or 1 hr in incubator (5%CO₂, 37°C).
   NOTE: For most cell types, 30 minutes incubation is adequate.
5. Measure the fluorescence on fluorescence plate reader at excitation wavelength set at 485 nm and emission wavelength at 530 nm.

RELATED PRODUCTS
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http://info2.gbiosciences.com/complete-bioassay-handbook

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