Calcein AM Cell Viability Assay

(Cat. # 786-1387)

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 (Fig.1) 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 Cell Viability Assay is more robust than tetrazolium salts or AlamarBlue® Dye, as the cells can be stained and quantified in less than 2 hrs.

Calcein AM Cell Viability Assay 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.

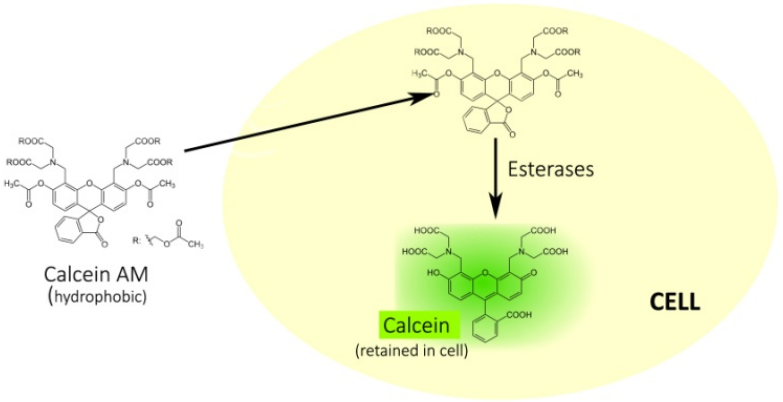


Fig.1: Hydrolysis of calcien AM dye to fluorescent calcein by cellular esterases

ITEM(S) SUPPLIED

Description	**Cat. # 786-1387 1000 assays**
Calcein AM Dye	4 vials
DMSO [Sterile Filtered]	250 µl

STORAGE CONDITIONS

The kit is supplied at ambient temperature. Store the kit at -20°C upon receiving. When stored and used as directed the kit is stable for 1 year.

IMPORTANT INFORMATION

- The stock solutions of Calcein AM dye prepared after adding DMSO should be stored at -20°C in preferably one time use aliquots.
- 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 Calcein 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.

ADDITIONAL ITEMS REQUIRED

- 96- Well black plate with clear flat bottoms.
- Fluorimeter
- 1x hanks salt solution and 20 mM HEPES or buffer of choice

PREPARATION BEFORE USE

1. Bring the kit components to room temperature.
2. Add 50 µl of DMSO per vial of Calcein AM Dye and mix well to get 1 mM stock solution. Use immediately or store the solution into one time use aliquots at -20°C.
3. 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: *Calcien 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%CO_2, 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

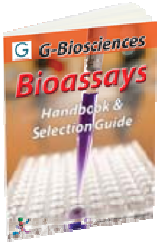
1. Plate cells in 96-well black walled cell culture plates in duplicate set. Leave the cells overnight in incubator (37°C, 5%CO_2) to adhere.
   NOTE: *The optimal seeding density should be determined by end user by plotting titration cell density curve for linear range and assay suitability for a cell 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 1hr in incubator (5%CO_2, 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.

TROUBLESHOOTING

Issue	Suggested reason	Possible solution
Low Fluorescence	Low concentration of Calcien AM used	Increase concentration of Calcein AM used
	96-well plate not compatible with Fluorimeter	Use black walled plates
	Cells not healthy during calcein AM incubation	Check health of cells during calcein AM incubation with Trypan blue.
Poor replicates or triplicates	Bubbles in wells	Pipette carefully avoiding bubble formation in wells
	Cells not pipette accurately	Resuspend cells evenly before pipetting into the wells
	Cells lost during wash step	Ensure no loss in wash step
High background fluorescence or high fluorescence	Calcein AM working solution not fresh	Prepare fresh Calcein AM working solution
	Cells not washed for removal of cell culture medium (containing serum)	Increase number of washed to ensure removal cell culture medium before incubation with Calcein AM
	Cell density to high	Decrease the number of cells added per well
	Incubation time with Calcein AM too long	Shorten the incubation time with Calcein AM

RELATED PRODUCTS

Download our Bioassays Handbook.



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