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ab270788 Calcein AM Cell Viability Assay Kit

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Calcein AM Cell Viability Assay Kit datasheet:
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1. Overview

The Calcein AM Cell Viability Assay Kit allows for easy and simultaneous differentiation of live and dead cells within a single sample.

The large quantum yield of Calcein dyes enables them to be readily detected within widely used applications such as flow cytometers and fluorescence microscopes. The degree of fluorescence correlates with relative cell viability status. For microscopy usage, Hoechst 33342 is included with the kit to concurrently label nuclei after labeling with Calcein. Because Calcein alone will detect cells that are alive, but some of which could possess compromised cell membrane structure and thus be in the process of dying, it is possible to obtain an overly positive picture of the overall health status of the cell population.

Sample size and Calcein AM staining concentration needed may vary based on the experimental conditions and the instrument used for analysis. Each investigator should adjust the amount of Calcein AM to accommodate the particular cell line and research conditions.

NOTE: Tests per vial range from 25 to 250 depending on application.

2. Materials Supplied and Storage

Store Calcein AM Reagent at -20°C and the rest of the kit at +4°C on receipt.

Δ Note: Once reconstituted with DMSO, use Calcein AM immediately, or store at ≤-20°C for up to 6 months, protected from light and thawed no more than twice during that time.

Item	Quantity	Storage temperature
Calcein AM Reagent	1 vial	-20°C
Hoechst 33342	1 vial	+4°C
10X Cellular Assay Buffer	1 bottle	+4°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- DMSO, up to 250 µL (50 µL per vial to reconstitute Calcein AM and more to create controls)
- DiH_2O, 540 µL per bottle to dilute 10X Cellular Assay Buffer
- Phosphate buffered saline (PBS) pH 7.4, 100 mL, to dilute Calcein AM and handle cells
- Cultured cells treated with the experimental conditions ready for staining
- FBS and/or BSA to add to the buffer when handling cells
- Cultured cells treated with the experimental conditions ready to be analyzed
- 90% ETOH or 3% formaldehyde to create live/dead controls for Calcein AM
- Hemocytometer
- Centrifuge at <200 *x g*
- FACS tubes (for flow cytometry analysis)
- Slides and coverslips
- Ice bath (if using 3% formaldehyde to create dead cell control population)
- Fluorescence microscope
- Flow cytometer
- Black 96-well microtiter plate, flat bottom, non-treated, non-sterile. If using a bottom reading instrument, use a plate with black walls and a clear bottom. If culturing cells in the plate, use a black sterile tissue culture plate. Plates only required for fluorescence plate reader analysis.

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Experimental Preparation

5.1 Staining cells with Calcein AM can be completed within a few hours. However, Calcein AM is used with living cells, which require periodic maintenance and cultivation several days in advance. In addition, once the proper number of cells has been cultivated, time must be allotted for the experimental treatment, which may vary. The recommended sample size is 400 µL cells at 5 x 10^5 cells/mL.

5.2 Create cell populations, such as:

a. Cells that were exposed to the experimental treatment.
b. A negative control population of cells that received a placebo treatment.

5.3 Culture cells to a density optimal for the specific experimental protocol. Cell density should not exceed 10^6 cells/mL. Cells cultivated in excess of this concentration may begin to naturally enter apoptosis. An initial experiment may be necessary to determine how much Calcein AM to use.

6. Controls

Create experimental samples and control cell populations:

- Treated experimental population(s): cells exposed to the experimental condition(s).
- **Positive control for Calcein AM (Live cells):** non-treated cells grown in a normal culture environment.
- **Negative control for Calcein AM (Dead cells):** cells treated with ethanol or formaldehyde to create a dead cell population. For example, briefly expose cells to 90% ethanol for 30-60 seconds at 37°C, or expose cells to 3% formaldehyde for 30 minutes on ice, then wash the cells 1X with PBS, and resuspend the sample in Cellular Assay Buffer

  Δ Note: A common pool of cells should be used to generate the positive and negative control populations for Calcein. These should contain similar quantities of cells. For example, if labeling with Calcein AM make 4 populations:

Control #	
1 and 2	Unlabeled: Live and dead cells
3 and 4	Calcein AM-labeled: Live and dead cells

7. Preparation of Calcein AM

7.1 Calcein AM is supplied as a lyophilized powder that may be slightly visible as an iridescent sheen inside the vial. Protect from light and use gloves when handling. Once diluted in aqueous buffer, Calcein AM solution must be used immediately; prepare it just before staining.

7.2 Reconstitute the vial of Calcein AM with 50 µL DMSO to form the stock solution at 2 mM. The stock solution should be colorless to light yellow. Once reconstituted in DMSO, it may be aliquoted and stored at ≤-20°C for 6 months protected from light. Avoid repeated freeze/thaw cycles.

7.3 Immediately prior to addition to the samples and controls, dilute the 2 mM Calcein AM stock solution 1:5 by adding 200 µL PBS. This creates a 400 µM Calcein AM solution. If staining at a final concentration of 10 µM, then this solution is ready to use.

7.4 If staining at a final concentration of 1 µM, further dilute the 400 µM Calcein AM solution 1:10 in PBS. The resulting solution is ready to use.

7.5 These amounts are recommendations, however, the sample size and Calcein AM staining concentration needed may vary based on the experimental conditions and the instrument used for analysis. Each investigator should adjust the amount of Calcein AM to accommodate the particular cell line and research conditions

8. Preparation of 1Xcellular Assay Buffer

- Cellular Assay Buffer is an isotonic solution used to stabilize cells when staining with Calcein AM. It contains mammalian proteins to stabilize cells, and sodium azide to retard bacterial growth (1X Cellular Assay Buffer contains 0.01% w/v sodium azide).
- Alternative solutions including cell culture media containing FBS and other additives may be used to stain cells instead of the 1X Cellular Assay Buffer.

8.1 10X Cellular Assay Buffer may form precipitates during cold storage. If this happens, gently warm it until all crystals have dissolved. Do not boil.

8.2 Dilute 10X Cellular Assay Buffer 1:10 in diH_2O. For example, add 60 mL 10X Cellular Assay Buffer to 540 mL diH_2O for a total of 600 mL.

8.3 1X Cellular Assay Buffer may be stored at 2-8°C and used within 1 week or frozen and used within 6 months.

9. HOECHST 33342

Hoechst 33342 is a cell-permeant nuclear stain that emits blue fluorescence when bound to double stranded DNA. It is used to stain the nuclei of living or fixed cells, to distinguish condensed pyknotic nuclei in apoptotic cells, and for cell cycle studies.

Hoechst 33342 is provided ready-to-use at 200 µg/mL. Hoechst 33342 can be used with Calcein AM to label the nuclei of live, dying, and apoptotic cells.

When bound to nucleic acids, it has a maximum absorbance at 350 nm and a maximum emission at 480 nm. It is revealed under a microscope using a UV-filter with excitation at 365 nm and emission at 480 nm.

10. Cell staining protocol

10.1 Prepare experimental and control cell populations. Ideally, the cell concentration should be 3-5 x 10^5 cells/mL. The concentration should not exceed 10^6 cells/mL, as cells cultivated in excess of this concentration may begin to naturally enter apoptosis. Just prior to staining, cells may need to be concentrated to 2-5 x 10^6 cells/mL as microscopy analysis methods require high cell concentrations. Start with a larger volume of cells at 3-5 x 10^5 cells/mL (which is a typical density for cell culture) and then concentrate cells and resuspend to 390 µL per sample when ready for Calcein AM staining.

10.2 Expose cells to the experimental or control condition.

- If analyzing with a flow cytometer, set aside two populations to create instrument controls with Calcein AM positive and negative controls.
- If analyzing with a fluorescence microscope, concentrate cells to 2-5 x 10^6 cells/mL just prior to Calcein AM staining. Fluorescence microscopy may require an excess of 2 x 10^6 cells/mL to obtain 5-20 cells per image field. Flow cytometry can analyze samples at 3-5 x 10^5 cells/mL.

10.3 Transfer 390 µL cells into fresh tubes.

10.4 To stain at 10 µM, add 10 µL of the reconstituted Calcein AM solution that had been diluted 1:5 in PBS to 390 µL cells, forming a final volume of 400 µL. To stain at 1 µM, add 10 µL of the Calcein AM solution that had been further diluted 1:10 in PBS to 390 µL cells, forming a final volume of 400 µL. If different cell volumes were used, add Calcein AM appropriately. Mix by gently flicking the tubes.

10.5 Incubate cells at 37°C protected from light. The incubation period may range from 30 minutes to several hours and should be optimized for each cell line and experimental condition.

Δ **Note:** As cells may settle on the bottom of the tubes, gently resuspend them by swirling cells every 20 minutes to ensure an even distribution of Calcein AM throughout the staining process.

Δ **Note**: Wash steps are not required, as any Calcein AM that has not been cleaved by intracellular esterases will be non-fluorescent.

11. Microscopy analysis

Follow Section 10.

11.1 Optional Hoechst 33342 staining: If dual staining with Hoechst, add Hoechst at 0.5% v/v and incubate for 5 minutes at 37°C. For example, add 2 µL of Hoechst to a 400 µL suspension cell sample.

11.2 When ready to view cells, place 1 drop of cell suspension onto a microscope slide and cover with a coverslip.

11.3 Observe cells under a fluorescence microscope using excitation at 470-490 plus a >520 nm long pass filter. Live cells fluoresce green. Observe Hoechst staining using a UV-filter with excitation at 365 nm and emission at 480 nm.

12. Flow cytometry Analysis

Follow Section 10.

12.1 Samples are ready for analysis. No further processing is required. To analyze the samples, measure green fluorescence (Calcein) on the FL-1 channel.

12.2 Live cells fluoresce green due to the presence of active esterases capable of cleaving Calcein AM.

12.3 Dead cells do not fluoresce green due to the absence of active esterases, leaving Calcein AM in its uncleaved (and non-fluorescent) form.

13. Fluorescence Plate reader Analysis

Follow Section 10

13.1 Determine the concentration and compare the cell density of each sample. The live/mock-treated population may have more cells than the killed population. Adjust the volume of the cell suspensions to equalize the cell density. When ready to read, cells should be between 2-5 x 10^6 cells/mL.

13.2 Pipette 100 µL cell suspension per well into a black-opaque-well, fluorescence plate reader-compatible, microtiter plate. It is recommended that each sample be tested in duplicate (at a minimum).

13.3 Do NOT use clear plates. Avoid bubbles.

13.4 Perform an endpoint read. If possible, set the excitation wavelength to 494 nm and the emission wavelength to 517 nm.

13.5 Live cells fluoresce green due to the presence of active esterases capable of cleaving Calcein AM.

13.6 Dead cells do not fluoresce green due to the absence of active esterases, leaving Calcein AM in its uncleaved (and non-fluorescent) form.

14. Notes

Technical Support

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