Version 3 Last updated 17 April 2024

ab270789 Live and Dead Cell Assay Kit (Calcein AM, 7-AAD)

View ab270789 Live and Dead Cell Assay Kit (Calcein AM, 7-AAD) datasheet:

www.abcam.com/ab270789

(use www.abcam.cn/ab270789 for China, or www.abcam.co.jp/ab270789 for Japan)

This product is for research use only and is not intended for diagnostic use.

Copyright © 2024 Abcam. All rights reserved

Table of Contents

Copyright © 2024 Abcam. All rights reserved

1. Overview

The Live and Dead Cell Assay Kit (Calcein AM, 7-AAD) kit combines Calcein AM with 7-aminoactinomycin D (7-AAD) to allow for easy and simultaneous labeling of live, membrane compromised, 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 and 7-AAD 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	99.5 µg	-20°C
7-Aminoactinomycin D (7-AAD) vital dye	2 vials	+4°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, 1,000 µL to reconstitute Calcein AM and 7-AAD
- DiH_2O, 540 µL per bottle to dilute 10X Cellular Assay Buffer
- Phosphate buffered saline (PBS) pH 7.4, 100 mL
- 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 and 7-AAD staining
- Hemocytometer
- Centrifuge at <200 *x g*
- FACS tubes (for flow cytometry analysis)
- Fluorescence microscope
- Flow cytometer
- Ice bath (if using 3% formaldehyde to create dead cell control population)
- Hot water bath (if using 56°C water bath to create dead cell control population)

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 and 7-AAD can be completed within a few hours. However, Calcein AM and 7-AAD are 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.
- The Calcein AM positive control also serves as the negative control for 7-AAD.
- **Positive control for 7-AAD (Dead cells):** cells treated with ethanol, formaldehyde, or heat to create a dead cell population (Section 9 and Figure 2).
- The 7-AAD positive control also serves as the negative control for Calcein AM.

Δ Note: A common pool of cells should be used to generate the positive control population for Calcein AM (which is also the negative control for 7-AAD) and the negative control population for Calcein AM (which is also the positive control for 7-AAD) and should contain similar quantities of cells. For example, if labeling with Calcein AM and 7-AAD stain, make 8 populations:

Control #	
1 and 2	Unlabeled: Live and dead cells
3 and 4	Calcein AM-labeled: Live and dead cells
5 and 6	Calcein AM- and 7-AAD-labeled: Live and dead cells
7 and 8	7-AAD-labeled: Live and dead cells

6.1 Preparation of dead cells (7-AAD Positive control/Calcein AM Negative control)

Prior to commencing the experiment, determine a reproducible method for obtaining a population of dead/killed cells to use as a positive control for 7-AAD staining. This can easily be achieved using many different techniques. For example, briefly expose cells to 90% ethanol for 30-60 seconds at 37°C, expose cells to 3% formaldehyde for 30 minutes on ice, or incubate cells in a 56°C hot water bath for 45 minutes.

7. Preparation of Calcein AM

Calcein AM optimally excites at 494 nm and has a peak emission at 517 nm (use FL-1 channel). Some flow cytometers may require use of a FL-1 99% attenuation filter.

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 1X Cellular Assay Buffer

- Cellular Assay Buffer is an isotonic solution used to stabilize cells when staining with Calcein AM and 7-AAD. 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. Preparation of 7-AAD

Detection of cell membrane integrity loss, indicative of necrosis or late stage apoptosis, is detected using the red fluorescent live/dead stain, 7-AAD. This vital dye works by penetrating cell membrane-compromised cells and tightly binding to GC rich regions of DNA.

7-AAD is supplied as a lyophilized powder that may be slightly visible as a red sheen inside the vial. Protect from light and use gloves when handling.

7-AAD optimally excites at 546 nm. It has a peak emission at 647 nm (use FL-3 channel), but displays adequate emission properties when excited within a blue (488-492 nm) light source.

9.1 Reconstitute each vial of 7-AAD with 260 µL DMSO to create a stock concentrate at 1 mg/mL. Mix by swirling or tilting the vial, allowing the DMSO to travel around the base of the amber vial until the reagent is completely dissolved. At room temperature, the reagent should be dissolved within a few minutes forming a red solution.

9.2 If storing the stock concentrate for future use, prepare small aliquots (50 µL, for example) to avoid freeze-thaw cycles. The stock concentrate will be stable for 6 months when protected from light and stored at or below -20°C.

9.3 When ready to stain cells, use 7-AAD at 1:200 dilution. For example, add 2 µL per 400 µL cells.

10. 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 and 7-AAD 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.

We suggest the addition of Hoechst at 0.5% v/v and an incubation time of 10-20 minutes .

11. Cell staining protocol

11.1 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 with Cellular Assay Buffer when ready for Calcein AM staining.

11.2 Expose cells to the experimental or control condition.

- If analyzing with a flow cytometer, set aside four populations to create instrument controls with Calcein AM positive and negative controls, and 7-AAD-positive and 7-AAD-negative cells.
- 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.

11.3 Transfer 390 µL cells into fresh tubes.

11.4 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.

11.5 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.

11.6 Stain with 7-AAD at a final concentration of 5 µg/mL (4 µM). This can be accomplished by:
- Adding the stock solution directly to the cell culture at 1:200; e.g. add 2 µL stock to 400 µL cell suspension.
- Or by diluting the stock concentrate 1:10 to form the working solution, and then adding the working solution to the cells at 1:20. For example:
- Add 50 µL 7-AAD stock concentrate to 450 µL PBS or sterile media to form the working solution.
- Mix by inverting or vortexing the vial at RT.
- Store on ice up to 2 hours.
- Add the working solution to the cell suspension at 1:20; e.g. add 20 µL diluted 7-AAD working solution into 380 µL cell suspension

11.7 Incubate for 10-30 minutes on ice while protecting from light.

12. Microscopy analysis of adherent cells

Calcein AM is not compatible with fixative. View stained cells immediately.

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

12.2 Observe cells under a fluorescence microscope using excitation at 470-490 plus a >520 nm long pass filter.

12.3 Live cells fluoresce green.

12.4 Necrotic or dead cells containing nucleic acid-bound 7-AAD fluoresce red.

12.5 Live cells with compromised membrane integrity fluoresce green and red.

13. Flow cytometry Analysis

13.1 To address compensation issues and set up the flow cytometer, prepare 2 instrument control populations (live and dead):

Cells stained with only Calcein AM.

- Live cells (untreated).
- Killed cells (cell membrane compromised).

Cells stained with only 7-AAD.

- Live cells (untreated).
- Killed cells (cell membrane compromised).

13.2 These controls are needed to adjust the instrument PMT's to separate 7-AAD-positive from 7-AAD-negative samples and to compensate for bleed-over of the red 7-AAD signal from FL-3 into FL-1. They will also help to clearly differentiate the Calcein-positive population from the Calcein AM-negative population and compensate bleed-over of the green Calcein signal from FL-1 into FL-3.

13.3 Set up the instrument compensation.

- Read the 7-AAD-positive and 7-AAD-negative controls to compensate bleed-over of the red 7-AAD signal from FL-3 into FL-1.
- Read the Calcein-positive and Calcein AM-negative controls to compensate bleed-over of the green Calcein signal from FL-1 into FL-3.

13.4 To read the samples for bicolor analysis:

- Measure green fluorescence (Calcein) on the FL-1 channel.
- Measure red fluorescence (7-AAD) on the FL-3 channel.
- Generate a log FL-1 versus log FL-3 dot plot.
- This will reveal 4 populations of cells:

1. Live cells fluoresce green due to the presence of active esterases capable of cleaving Calcein AM, but do not fluoresce red as their intact cell membranes exclude 7-AAD.
2. Live; membrane-compromised cells: fluoresce green due to the presence of active esterases capable of cleaving Calcein AM, and fluoresce red due to compromised membrane integrity allowing 7-AAD to enter cell and intercalate with nucleic acids.
3. Dead; red fluorescing cells are 7-AAD positive dead cells. These cells lack active esterases and have compromised cell membranes.

4. Unstained cells, no fluorescence. Few cells are expected to fall into this population. A high percentage of cells in the lower left quadrant could indicate that a problem occurred during the staining process such as too low of a staining concentration.

14. Notes

Technical Support

Copyright © 2024 Abcam. All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print.

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)

Copyright © 2024 Abcam. All rights reserved