Calcein AM/PI Mammalian Cell Viability Assay Kit

Catalog No.: abx295119

Size: 500 tests

Storage: Store all components at -20 °C in the dark.

Application: For the diffferentiation of living and dead mammalian cells with esterase activity.

Introduction

Abbexa's Calcein AM/PI Mammalian Cell Viability Assay Kit is a quick, convenient, and sensitive method for differentiating between living and dead cells with esterase activity. A cell-permeable non-fluorescent fluorescein, Calcein AM, is catalysed by cytosolic esterase to produce the cell-impermeable green fluorescent probe Calcein. Dead cells show decreased esterase activity so do not produce Calcein as strongly. Furthermore, following cell death, there is a loss of selective membrane permeability and the red fluorescent probe Proidium Iodide (PI) can enter cells and bind to double-stranded DNA. Live cells will therefore show green fluorescence (494/517 nm), and dead cells show red fluorescence (535/617 nm).

Kit components

1. Calcein AM Solution (100 µM): 500 µl
2. PI Solution (750 µM): 500 µl
3. Calcein AM Buffer: 2 × 55 ml

Materials required but not provided

1. Fluorescence microscope
2. Flow cytometer
3. PBS (pH 7.2 – 7.4)
4. Pipette and pipette tips
5. Sterile centrifuge tubes
6. Centrifuge
7. Vortex mixer
8. Incubator
9. Glass slides
10. 96-well microplate

Flow Cytometery

A. Preparation of samples and reagents

Allow all reagents to equilibrate to room temperature and vortex mix before use.

1. **Samples:** Centrifuge cells at 300 × g for 5 minutes. Discard supernatant and resuspend cells by adding 1 ml of PBS (pH 7.2 – 7.4). Centrifuge again at 300 × g for 5 minutes. Discard supernatant, wash then discard supernatant again.
2. **Calcein AM/PI working solution:** Prepare enough for the amount of sample tested. The recommended ratio is 200 µl per 1 – 5 × 10^5 cells. Prepare the solution as summarized in the following table:

Component	Voulme
Calcein AM Solution (100 µM)	1 µl
PI Solution (750 µM)	10 µl
Calcein AM Assay Buffer	10 ml

Note:

- Prepare working solution fresh for immediate use
- For Calcein AM single staining, do not include PI solution in working solution
- For PI single staing, do not include Calcein AM solution in working solution

B. Assay Procedure

Pre-heat the incubator and ensure it has reached a stable temperature before use.

1. Label sterile tubes for each sample and control. *It is strongly recommended to prepare all the tubes in duplicate.*
2. In sample tubes, resuspend cells by adding 200 µl of working solution per every 1- 5 × 10^5 cells.
3. Add 200 µl of calcein AM Assay Buffer to control tubes.
4. Incubate at room temperature for 5 – 15 minutes in the dark.
5. Perform flow cytometry. If this cannot be carried out immediately, store sample at 4°C for up to 2 hours in the dark.

Note:

- Calcein can be detected using a FITC channel whilst PI can be detected using a PE or Percp/Cy5.5 channel.

Fluorescence Microscopy

A. Preparation of samples and reagents

Allow all reagents to equilibrate to room temperature and vortex mix before use.

1. **Adherent cell samples:** Carefully remove the culture medium. Wash cells with PBS (pH 7.2 – 7.4). Carefully remove PBS. Repeat the wash step once.

   Suspended cell samples: Centrifuge cells at 300 × g for 5 minutes. Discard supernatant and resuspend cells by adding 1 ml of PBS (pH 7.2 – 7.4). Centrifuge again at 300 × g for 5 minutes. Discard supernatant, wash then discard supernatant again.

2. **Calcein AM/PI working solution:** Prepare enough for the amount of sample tested. For adherent cells, use 100 µl For suspended cells, the recommended ratio is 200 µl per 1 – 5 × 10^5 cells. Prepare the solution as summarized in the following table:

Component	Volume
Calcein AM Soultion (100 µM)	10 µl
PI Solution (750 µM)	10 µl
Calcein AM Assay Buffer	1 ml

Note:

- Prepare working solution fresh for immediate use
- If adherent cells are easily detached, it is recommended to replace the Calcein Am Assay Buffer with basic culture medium

C. Assay Procedure

Adherent cells

Pre-heat the incubator and ensure it has reached a stable temperature before use.

1. Label sample and control wells. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 100 µl of working solution to each well.
3. Incubate at 37 °C for 10 – 30 minutes. If basic medium has been used, increase incubation time to 30 – 60 minutes
4. Analyze sample using a fluorescence microscope. (Calcein is green fluorescent 494nm/517nm, PI is red fluorescent 535nm/617nm).

Suspended cells

Pre-heat the incubator and ensure it has reached a stable temperature before use.

1. Label each sample tube. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 200 µl of working solution per every 1- 5 × 10^5 cells.
3. Incubate at room temperature for 15 – 20 minutes.
4. Transfer cell suspension onto a glass slide.

5. Gently cover glass and analyze sample using a fluorescence microscope. (Calcein is green fluorescent 494nm/517nm, PI is red fluorescent 535nm/617nm)

Notes:

- This kit is intended for research use only
- Samples/sample media should not contain primary or secondary amines, as fatty histamines may lyse AM esters.
- Staining at 37°C can reduce the incubation time, staining at room temperature can reduce the effect of probe entry into organelles.
- Positive controls can be prepared by treating cells with 5 % – 20 % DMSO for 2 – 4 hours, or with 70 % Ethanol for 30 minutes.
- Avoid washing cells with buffers containing Mn^{2+} ions, as these will affect fluorescence performance.
- When released, Calein may be expelled from cells by Glycoprotein P activity, reducing staining performance.
- Samples with cell walls such as plant cells will prevent Calcein AM entry, and so are not suitable.

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.