EasyAssay™ Calcein AM Cell Viability Assay

(1000 Tests)

Zellbio GmbH (Germany)

CAT No. ZX-88105-1000

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Application:

Detection of Cell Viability, Proliferation & cytotoxicity

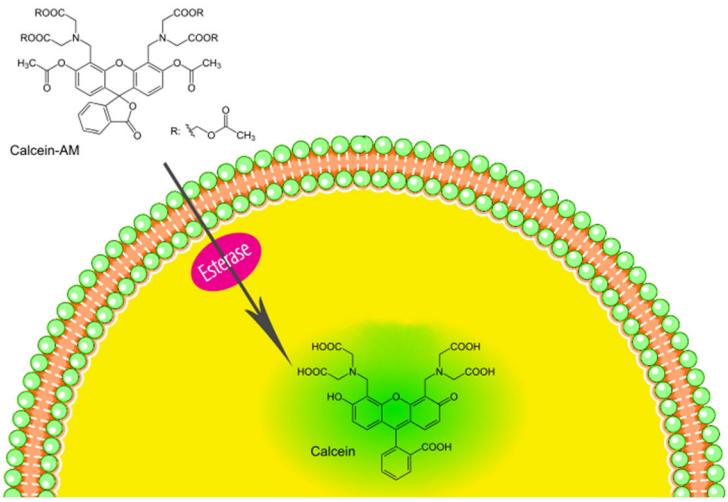
!!! Caution: This product is for Research Use Only. Not for *in-vitro* Diagnostics !!!

Table of Contents

Please read this insert completely prior to using the product.

Background

Calcein AM or Calcein acetoxymethyl ester is a non-fluorescent, hydrophobic compound, which permeates easily through cell membranes into live cells. Inside the cytoplasm, endogenous esterases hydrolyze the non-fluorescent calcein AM dye to calcein (highly negatively charged green fluorescent dye), which is retained in the cytoplasm in live cells. Hence, the intensity of calcein is directly proportional to the number of viable cells in culture media.



The calcein AM Cell Viability assay is a fluorescent assay that measures cellular viability based on conversion of the calcein AM compound to calcein. As the fluorescent Calcein only retain in live cells, the amount of The fluorescent signal generated due to calcein is proportional to the number of viable cells in the sample. Due to the interference of esterases and phenol red in the culture medium with the fluorescence measurement, it is mandatory to replace cell culture medium with PBS prior to applying the calcein AM. The conversion of calcein AM to calcein in the assay can be quantified by measuring the fluorescent intensity at 535 nm with the excitation at 485 nm.

Intended use

Determination of cell viability, cell proliferation and cytotoxicity.

Materials supplied in the Kit

Component	*Quantity*
Calcein AM	2 x 50 µg
PBS Tablet	1
DMSO	100 µL
0.2 µM Fliter	1

Storage instruction

All reagents should be stored at room temperature, except Calcein AM that must be stored at -20°C.

Materials required but not supplied

Distilled water (dH_2O)

Precision pipettes and disposable pipette tips

Sterile 96 well plate

Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

General remarks

- Equilibrate all kit components to room temperature (RT) before use.
- The instruction must be strictly followed.
- Pipette tips should not be used more than once to avoid cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.

Assay preparation

i. **1X PBS Solution:** Prepare 1X PBS solution by dissolving 1 provided small PBS tablet in 100 mL dH_2O. PBS solution can be stored at room temperature.

ii. **1000X Calcein AM Stock Solution:** Prepare Calcein AM stock solution by dissolving 50 µg Calcein AM (1 vial) in 50 µL DMSO (each vial can be used to run 500 assays). Calcein AM stock solution can be stored at -20°C and protected from light (use dark chamber or aluminum foil) up to 2 months.

iii. **1X Calcein AM Working Solution:** Prepare 1:1000 dilution of 1000X Calcein AM Stock Solution with 1X PBS (50 µL 1000X Calcein AM Stock Solution with 50 mL 1X PBS). 1X Calcein AM Working Solution must be used immediately after preparation.

Sample preparation

i. Cultivate cells into an appropriate 96-well plate containing 100 µL/well of cell culture medium. The optimal seeding density and the appropriate time of incubation may vary depending on the cell types and the desired chemical or physical treatments and must be evaluated by the end user.

ii. Each plate must be included at least two wells as background control which contain no cells.

- We strongly recommend to determine the optimal cell number and incubation time for your specific cells before performing a large number of Calvein AM assays.

Assay Procedure

1. **Optional**: treat the cells with compounds of interest dissolves in an appropriate solvent for desired time period. In the case of treatment include control wells for the solvent, the cells must be treated with the same solvent without compounds of interest.
2. Aspirate the medium from the wells (for suspension cells, spin the plate at 500-1000 g for 5 minutes at 4°C and carefully discard the media)
3. Add 100 µL of the 1X Calcein AM Working Solution to each well. Mix gently for one minute.
4. Incubate the cells for 30 minutes (adherent cells & suspension cells) at 37°C in a CO_2 incubator.
5. Read the fluorescent intensity at 530 nm with the excitation at 485 nm.

Calculations

i. Average the duplicate Fluorescent Unit (FLU) readings for each sample.

ii. Subtract the background absorbance from the signal absorbance to obtain normalized absorbance values.