Calcein AM Cell Viability Assay Kit (Fluorometric) (BN00563)

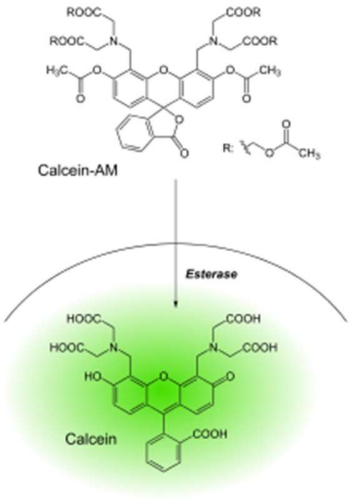
(Catalog BN00563, 1000 assays; Store kit at -20°C)

I. Introduction:

Quantification of number of viable cells is an indispensable tool in Cell Biology research. Assay Genie's Calcein AM Cell Viability Assay Kit is a fluorometric method for extremely sensitive quantification of viable cells that can detect as low as 50 viable cells in less than 30 min. Calcein AM is a non-fluorescent, hydrophobic compound that easily penetrates intact and live cells. Hydrolysis of Calcein AM by intracellular esterase produces a hydrophilic, strongly fluorescent compound that is retained in the cell cytoplasm and can be measured at Ex/Em = 485/530 nm. The measured fluorescence intensity is proportional to the number of viable cells. This assay kit provides an easy-to-use, non-radioactive, and high-throughput method for cell proliferation, cell viability, chemotaxis, cytotoxicity and apoptosis.

II. Applications:

- Measurement of cell proliferation in response to growth factors, cytokines, mitogens and nutrients
- Analysis of cytotoxic and cytostatic compounds such as anticancer drugs, toxic agents and other pharmaceuticals
- Assessment of physiological mediators and antibodies that affect cell growth



III. Sample Type:

- Adherent and suspension cells
- Proliferating and non-proliferating cells

IV. Kit Contents:

Components	BN00563	Cap Code
Calcein AM	2 x Vials	Green
Calcein Dilution Buffer	100 ml	NM

V. User Supplied Reagents and Equipment:

- 96-well plate with flat bottom. White plate is preferred for this assay
- Fluorescence plate reader
- DMSO

VI. Storage Conditions and Reagent Preparation:

Store kit at - 20°C, protected from light. Centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

- **Calcein AM:** Resuspend in 100 µl anhydrous DMSO (not provided) as needed. Aliquot and store -20°C. Use within 2 months.

VII. Calcein AM Cell Viability Assay protocol:

1. Grow cells at varying densities (100-500,000 cells per ml) in an appropriate plate according to the desired protocol. For adherent cells, carefully discard the media. For suspension cells, spin the 96-well plate at 1,000 X g for 5 min. at 4°C in a microplate compatible centrifuge and carefully discard the media. Dilute Calcein AM solution in Calcein Dilution Buffer 1:500 as needed (e.g. 1 µl Calcein AM dye in 499 µl of Buffer). Add 100 µl of freshly diluted Calcein AM solution to each well. Incubate at 37°C for 30 min & read fluorescence (Ex/Em = 485/530 nm).

   Notes:

   a. Clear plate can be used to ensure cell adherence, but background fluorescence may reduce assay sensitivity. If using clear plate, we recommend lysing cells after 30 min. incubation with Calcein AM. Carefully remove the media from each well & add 100 µl Cell Lysis Buffer. Incubate for 10 min. at room temperature & transfer cell lysates into a 96-well white plate. Measure fluorescence.

   b. Use diluted Calcein AM immediately. Don't store the diluted Calcein AM.

   c. Appropriate incubation time depends on the individual cell type and cell concentrations used. Therefore, it is recommended to determine the optimal incubation time for each experiment.

   d. We recommend washing the cells with 100 µl PBS to remove carry-over media and serum, as phenol red and serum may interfere with the sensitivity of the assay.

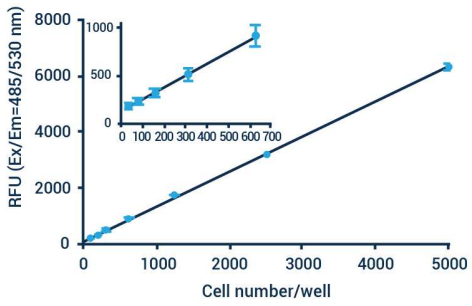


Figure: Cell Viability Assay: Fibroblast cells were grown in DMEM supplemented with 10% FBS, harvested using trypsin and counted using Trypan blue and a hemocytometer. Cells were serially diluted in a clear cell culture plate and incubated for 30 min. with Calcein AM at 37°C. After incubation, cells were lysed using Cell Lysis Buffer for 10 min. at room temperature. Cell lysates were transferred into a 96-well white plate & fluorescence was measured. Inset graph is an expanded segment of the assay data at lower cell number per well.

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