ELISA PRODUCT INFORMATION & MANUAL

Calcein AM Cell Viability Assay Kit (Fluorometric)

NBP2-54887

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

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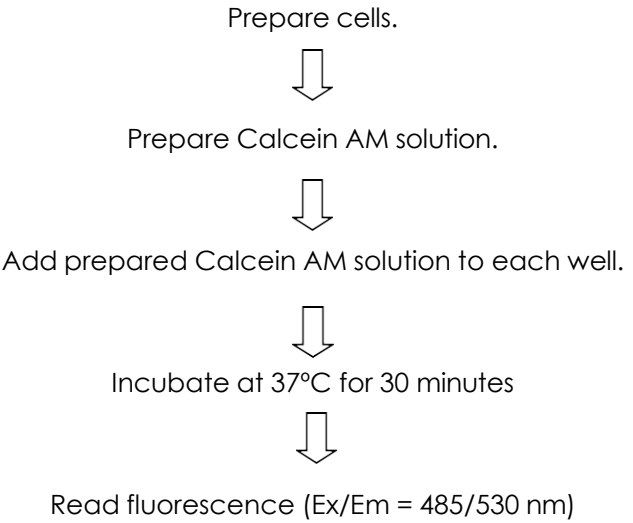
1. Overview

Calcein AM Cell Viability Assay Kit (Fluorometric) (NBP2-54887) is a simple, extremely sensitive quantitative assay to measure the viability of adherent and suspension cells that can detect as low as 50 viable cells in less than 30 minutes.

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.



2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Reconstituted components are stable for 2 months.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Calcein AM	2 x 1 vials	-20°C	-20°C
Calcein Dilution Buffer	100 ml	-20°C	-20°C

3. Materials Required, Not Supplied

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

- Microplate reader capable of measuring fluorescence at Ex/Em = 485/530 nm
- 96 well plate with clear flat bottom, preferably white (for fluorometric assay)

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

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

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Calcein AM:

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

5.2 Calcein Dilution Buffer:

Ready to use as supplied.

6. Sample Preparation

General sample information:

We recommend that you use fresh samples for the most reproducible assay. If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware, however, that this might affect the stability of your samples and the readings can be lower than expected. Avoid multiple freeze-thaws.

For certain samples, it may be advantageous to add protease inhibitors: we recommend Protease Inhibitor Cocktail II (ab201116) [AEBSF, aprotinin, E-64, EDTA, leupeptin] as a general use cocktail.

7. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

7.1 Preliminary:

1. Grow cells at varying densities (100-500,000 cells per ml) in an appropriate plate according to the desired protocol.
2. For adherent cells, carefully discard the media.
3. For suspension cells, spin the 96-well plate at 1,000 X g for 5 minutes at 4°C in a microplate compatible centrifuge and carefully discard the media.

7.2 Dilute Calcein AM solution in Calcein Dilution Buffer 1:500 as needed (e.g. 1 µl Calcein AM dye in 499 µl of Buffer).
7.3 Add 100 µl of freshly diluted Calcein AM solution to each well.
7.4 Incubate at 37°C for 30 minutes.
7.5 Read Fluorescence at Ex/Em = 485/530 nm.

7.6 Using a clear plate:

Clear plate can be used to ensure cell adherence but background fluorescence may reduce assay sensitivity.

1. Carefully remove medium.

2. Add 100 µL freshly diluted Calcein AM to each well and incubate for 30 minutes at 37°C.

3. Remove Calcein AM and add 100 µL cell lysis buffer. Incubate for 10 minutes at room temperature.

4. Transfer cell lysates into a 96-well white plate. Measure fluorescence.

Δ Note:
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.

Δ Note:
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.

8. Typical Data

Data provided **for demonstration purposes only**.

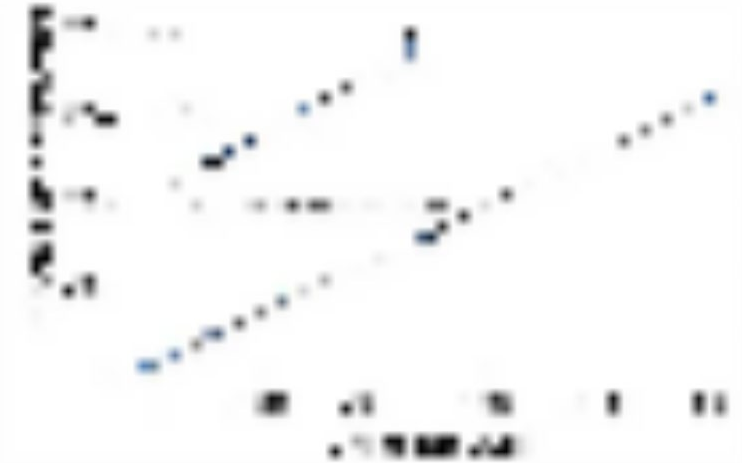


Figure 1: Cell Viability Assay: Fibroblast cells were grown in DMEM supplemented with 10% FBS, harvested using trypsin and counted using Trypan blue and a hemocytometer. Cells were serially diluted in a clear cell culture plate and incubated for 30 min. with Calcein AM at 37°C. After incubation, cells were lysed using Cell Lysis Buffer for 10 minutes at room temperature. Cell lysates were transferred into a 96-well white plate and fluorescence was measured. Inset graph is an expanded segment of the assay data at lower cell number per well.

9. Notes