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ab228556
Calcein AM Assay Kit (Fluorometric)

For the measurement of viability in adherent and suspension cells.

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

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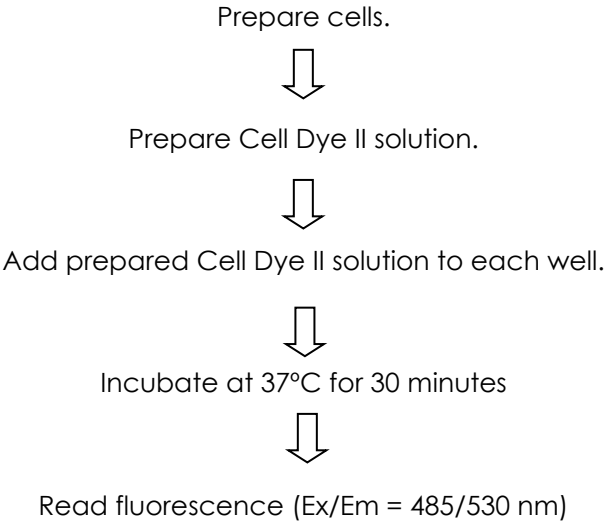
1. Overview

Calcein AM cell viability assay kit (fluorometric) (ab228556) 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.

Cell Dye II is a non-fluorescent, hydrophobic compound that easily penetrates intact and live cells. Hydrolysis of Cell Dye II 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)
Cell Dye II	2 x 1 vials	-20°C	-20°C
Assay Buffer 27	100 mL	-20°C	-20°C

PLEASE NOTE: Assay Buffer 27 was previously labelled as Assay Buffer XXVII and Calcein Dilution Buffer. The composition has not changed.

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 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. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Cell Dye II:

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

5.2 Assay Buffer 27:

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 Cell Dye II solution in Assay Buffer 27 Buffer 1:500 as needed (e.g. 1 µl Cell Dye II dye in 499 µl of Buffer).
7.3 Add 100 µl of freshly diluted Cell Dye II 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 Cell Dye II to each well and incubate for 30 minutes at 37°C.

3. Remove Cell Dye II 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**.

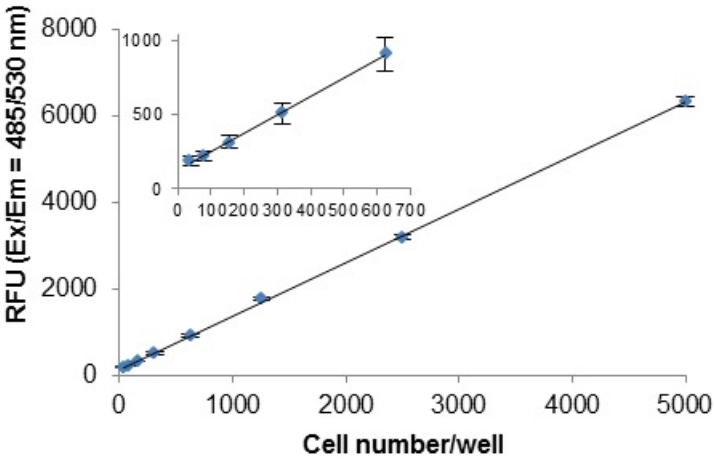


Figure 1: Cell Viability Assay: Fibroblast cells were grown in DMEM supplemented with 10% FBS, harvested using trypsin and counted using Trypan blue and a hemocytometer. Cells were serially diluted in a clear cell culture plate and incubated for 30 min. with Cell Dye II at 37°C. After incubation, cells 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

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)