Calcein AM Cell Viability Assay

Catalog Number: 4892-010-K

1000 tests

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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Manufactured and Distributed by:

USA R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413
TEL: 800 343 7475 612 379 2956
FAX: 612 656 4400
E-MAIL: info@bio-techne.com

Distributed by:

Europe | Middle East | Africa Bio-Techne Ltd.
19 Barton Lane, Abingdon Science Park
Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449
FAX: +44 (0)1235 533420
E-MAIL: info.emea@bio-techne.com

China Bio-Techne China Co., Ltd.
Unit 1901, Tower 3, Raffles City Changning Office,
1193 Changning Road, Shanghai PRC 200051
TEL: +86 (21) 52380373 (400) 821-3475
FAX: +86 (21) 52371001
E-MAIL: info.cn@bio-techne.com

INTRODUCTION

The Calcein AM Cell Viability Assay provides a simple, rapid, and accurate method to measure cell viability and/or cytotoxicity. Calcein AM is a non-fluorescent, hydrophilic compound that easily permeates intact, live cells. The hydrolysis of Calcein AM by intracellular esterases produces calcein, a hydrophilic, strongly fluorescent compound that is well-retained in the cell cytoplasm. Cells cultured in black-walled plates can be stained and quantified in less than two hours.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Do not mix or substitute reagents with those from other lots or sources.
- Variations in sample collection, processing, and storage may cause sample value differences.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between sample and reagent additions.

PRECAUTION

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

MATERIALS PROVIDED & STORAGE CONDITIONS

Use within 3 months from date of receipt.

PART	PART #	AMOUNT PROVIDED	STORAGE OF UNOPENED MATERIAL
Calcein AM	4892-010-01	2 vials (50 µg/vial)	Store ≤ -20 °C*.
10X Calcein AM DW Buffer	4892-010-02	200 mL bottle	Store at room temperature.

* Desiccate and protect from light

OTHER MATERIALS/EQUIPMENT REQUIRED BUT NOT SUPPLIED

- Fluorescence plate reader equipped with a 490 nm excitation filter and a 520 nm emission filter.
- Pipettes and tips.
- Deionized sterile water.
- Black-walled culture plates. Depending on cell type and density, it may be possible to use transparent plates. However, background fluorescence may significantly reduce assay sensitivity. See the manufacturer's recommendations for your fluorometer and empirically test the use of transparent plates with your system.
- Cell culture media, supplies, and centrifuge equipped to handle microplates (centrifuge able to handle microplates is ideal but optional).
- Anhydrous DMSO.
- Equipment to desiccate at ≤ -20 °C.

REAGENT PREPARATION

1X Calcein AM DW Buffer - Dilute 10X Calcein AM DW Buffer 1:10 in deionized sterile water to make 1X Calcein AM DW Buffer. For each 96-well plate, use 5.0 mL of 10X Calcein AM DW buffer and 45 mL of deionized sterile water.

Calcein AM Stock Solution- The molecular weight of Calcein AM is 995 gm/mole. Resuspend the dehydrated pellet of one tube (50 µg) in 25 µL of anhydrous DMSO to make a 2 mM Calcein AM Stock Solution. Return the unused portion of the Calcein AM Stock Solution to storage at ≤ -20 °C under desiccation.

2X Calcein AM Working Solution - Immediately prior to use, dilute the Calcein AM Stock Solution in 1X Calcein AM DW Buffer to a 2X Calcein AM Working Solution. Prepare enough for all wells using 50 µL/well at the appropriate concentration. For example, for one 96-well plate using a 1 µM final concentration of Calcein AM: dilute 5.0 µL of the Calcein AM Stock Solution in 5.0 mL of 1X Calcein AM DW Buffer to make a 2X Calcein AM Working Solution (2 µM). Diluted Calcein AM must be used immediately, as it will hydrolyze to calcein in solution.

Note: *The final concentration of the Calcein AM will need to be empirically determined for different cell types and/or experimental conditions; ranges of 1 µM to 10 µM have been reported.*

ASSAY PROTOCOL

PREPARATION OF SUSPENSION CELLS

1. Seed cells at varying densities (1 x 10^3 - 5 x 10^5 cells/mL) in appropriate cell culture medium in black-walled plates. Treat cells according to the experimental protocol (varying amounts of proliferative or toxic compounds, etc.).
   Note: *Alternatively, cells can be grown in transparent plates, and transferred to black-walled plates for reading.*
   Note: *For conversion of RFU to cell number, the range of cell concentrations needed for a standard curve may need to be optimized to ensure the best dynamic range.*
2. Centrifuge at 250 x g for 5 minutes with a centrifuge equipped to handle 96-well plates. Alternatively, transfer cells to microfuge tubes for centrifugation and return to the plate to read.
3. Carefully discard the media supernatant and add 100 µL of 1X Calcein AM DW Buffer.
4. Centrifuge at 250 x g for 5 minutes.
5. Remove the 100 µL of 1X Calcein AM DW Buffer and replace with 50 µL of fresh 1X Calcein AM DW Buffer. It is important to remove any carry-over media in the supernatant, as phenol red and serum will interfere with the sensitivity of the assay.
6. Add 50 µL of freshly diluted 2X Calcein AM Working Solution to each well.
7. Incubate for 30 minutes at 37 °C under 5% CO_2 (or normal cell growth conditions).
8. Record fluorescence using a 490 nm excitation filter and a 520 nm emission filter. The fluorescence intensity is proportional to the number of viable cells

ASSAY PROTOCOL *CONTINUED*

PREPARATION OF ADHERENT CELLS

1. Seed cells at varying densities (1 x 10^3 -1 x 10^5 cells/mL) in appropriate cell culture medium in 96-well plates and treat according to experimental protocol (varying amounts of proliferative or toxic compounds).
   Note: *Transparent plates may also be used to ensure cell adherence but background fluorescence may reduce assay sensitivity.*

   Note: *The optimal range of cell number may need to be optimized to ensure the best dynamic range.*
2. Discard the media supernatant and add 100 µL of 1X Calcein AM DW Buffer.
3. Remove the 100 µL of 1X Calcein AM DW Buffer and replace with 50 µL of fresh 1X Calcein AM DW Buffer. It is important to remove any carry-over media, as phenol red and serum will interfere with the sensitivity of the assay.
4. Add 50 µL per well of freshly prepared 2X Calcein AM Working Solution.
5. Incubate for 30 minutes at 37 °C under 5% CO_2 (or normal culture conditions).
6. Record fluorescence using 490 nm excitation filter and a 520 nm emission filter. The fluorescence intensity is proportional to the number of viable cells.

DATA EXAMPLE

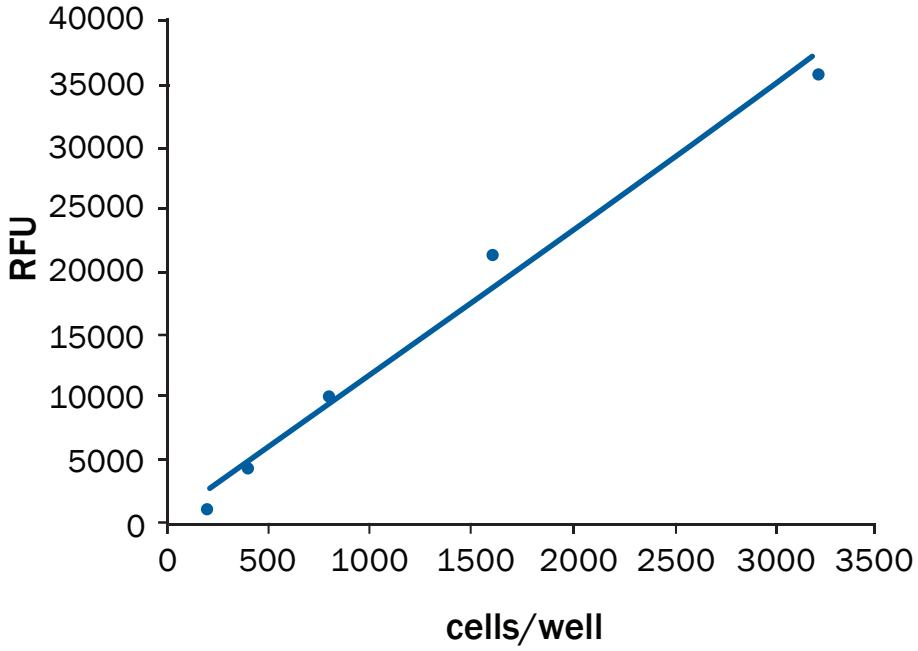

Figure 1. Calcein AM Quantification of Jurkat Cells - Jurkat cells were grown in RPMI supplemented with 10% Fetal Bovine Serum, washed with 1X Calcein AM DW Buffer, and counted using Trypan blue and a hemacytometer. Cells were serially diluted in a black-walled microplate and then incubated with 1 µM Calcein AM for 30 minutes at 37 °C under 5% CO_2. Fluorescence values were obtained using a 485 nm excitation filter and a 520 nm emission filter.

STANDARDIZATION

There are two methods for assay standardization:

1. **Measure relative differences or compare absolute cell number.** To monitor relative changes in cell number in the same cell type it is not necessary to calibrate the system. Data may be presented as the percent change in fluorescence intensity relative to an experimental control.

2. **Comparison of absolute cell number.** To calibrate using absolute cell number, determine the cell number in a sample and plate out dilutions in triplicate covering a range of 1×10^3 - 5×10^5 cells/mL in 50 µL of medium. Perform the standard assay. Determine averages of triplicate values and plot data as cell number per well vs. fluorescence intensity. To calibrate fluorescence values across 96-well plates, the same gain setting must be used.

TROUBLESHOOTING

PROBLEM	ACTIONS
Low fluorescence values	Increase concentration of Calcein AM used.
	Check health of cells during incubation with Calcein AM (using Trypan Blue).
	Incubate plate **in the dark.**
Poor triplicates	Ensure no bubbles are present in wells.
	Pipet cells accurately.
	Check accuracy of pipettor.
	Ensure no loss of cells during wash steps.
High background	Use black-walled plates.
	Use Calcein AM DW Buffer.
	Use freshly diluted Calcein AM.
	Increase washing to ensure media removal.
	Shorten incubation time with Calcein AM.
	Decrease number of cells per well.

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