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.
<table>
<thead>
<tr>
<th>Contents</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>2</td>
</tr>
<tr>
<td>LIMITATIONS OF THE PROCEDURE.</td>
<td>2</td>
</tr>
<tr>
<td>PRECAUTIONS</td>
<td>2</td>
</tr>
<tr>
<td>REAGENTS PROVIDED</td>
<td>3</td>
</tr>
<tr>
<td>MATERIALS REQUIRED BUT NOT SUPPLIED</td>
<td>3</td>
</tr>
<tr>
<td>REAGENT PREPARATION</td>
<td>3</td>
</tr>
<tr>
<td>ASSAY PROTOCOL</td>
<td>4</td>
</tr>
<tr>
<td>SAMPLE EXPERIMENTAL RESULTS</td>
<td>5</td>
</tr>
<tr>
<td>STANDARDIZATION</td>
<td>5</td>
</tr>
<tr>
<td>TROUBLESHOOTING GUIDE</td>
<td>6</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>7</td>
</tr>
</tbody>
</table>

**DISTRIBUTED BY:**

R&D Systems, Inc.
614 McKinley Place NE
Minneapolis, MN 55413
United States of America

TELEPHONE: (800) 343-7475  
FAX: (612) 379-2956  
E-MAIL: info@RnDSystems.com

R&D Systems Europe, Ltd.
19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB
United Kingdom

TELEPHONE: +44 (0)1235 529449  
FAX: +44 (0)1235 533420  
E-MAIL: info@RnDSystems.co.uk

R&D Systems GmbH
Borsigstrasse 7
65205 Wiesbaden-Nordenstadt
Germany

TELEPHONE: +49 (0)6122 90980  
FAX: +49 (0)6122 909819  
E-MAIL: infogmbh@RnDSystems.co.uk

R&D Systems Europe
77 boulevard Vauban
59041 LILLE CEDEX
France

FREEPHONE: +0800 90 72 49  
FAX: +0800 77 16 68  
E-MAIL: info@RnDSystems.co.uk
INTRODUCTION
The Calcein AM kit provides a simple, rapid, and accurate method to measure cell viability and/or cytotoxicity. Calcein AM is a non-fluorescent, hydrophobic 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 grown in black-walled plates can be stained and quantified in less than two hours.

Features/advantages of this assay include:
- Suitable for both proliferating and non-proliferating cells
- Ideal for both suspension and adherent cells
- Non-radioactive microplate
- Rapid (no solubilization step)
- Ideal for high-throughput assays
- Better retention and brightness compared to other fluorescent compounds (i.e. fluorescein)
- Useful in a variety of studies, including cell adhesion, chemotaxis, multi-drug resistance, cell viability, apoptosis, and cytotoxicity
- Suitable for use with a wide variety of techniques, including microplate assays (described here), immunocytochemistry, flow cytometry, and in vivo cell tracing

LIMITATIONS OF THE PROCEDURE
- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.

PRECAUTIONS
The physical, chemical, and toxicological properties of the products contained in this kit have not yet been fully investigated. The use of gloves, lab coats, and eye protection while using any of these chemical reagents is highly recommended. R&D Systems assumes no liability for damage resulting from handling or contact with these products. Material Safety Data Sheets are available upon request.
REAGENTS PROVIDED

Calcein AM (Part 4892-010-01) - 2 vials (50 µg per vial) containing Calcein AM. Store at ≤ -20° C in a manual defrost freezer. Desiccate and protect from light.

10X Calcein AM Dilution/Wash (DW) Buffer (Part 4892-010-02) - 200 mL of dilution/wash buffer. Store at 2 - 8° C.

MATERIALS REQUIRED BUT NOT PROVIDED

- Fluorescence plate reader equipped with a 490 nm excitation filter and 520 nm emission filter
- Centrifuge equipped to handle microplates (ideal, but not required)
- Equipment to desiccate at ≤ -20° C
- Black-walled culture microplates (R&D Systems, Catalog # DY991)
- Pipettes and pipette tips
- Deionized or distilled water
- Cell culture media
- 37° C CO₂ incubator
- Anhydrous DMSO

REAGENT PREPARATION

1X Calcein AM DW Buffer - Dilute the 10X Calcein AM DW Buffer to 1X before use. For each 96-well microplate, use 5 mL of 10X Calcein AM DW Buffer and 45 mL of deionized sterile water.

Calcein AM - The molecular weight of Calcein AM is 995 g/mole. Reconstitute one vial (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.

Immediately prior to use, dilute the Calcein AM Stock Solution in 1X Calcein AM DW Buffer to a 2X Calcein AM Working Solution, preparing enough for all wells using 50 µL per well at the appropriate concentration.

For example, for one 96-well microplate using a 1 µM final concentration of Calcein AM, dilute 5 µL of the Calcein AM Stock Solution in 5 mL of pre-warmed (22° C) 1X Calcein AM DW Buffer to make a 2 µM (2X) Calcein AM Working Solution. 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 - 10 µM have been reported.
ASSAY PROTOCOL

SUSPENSION CELLS

1. Grow cells at varying densities (1,000 - 500,000 cells per mL) in appropriate medium in black-walled microplates and treat according to experimental protocol (varying amounts of proliferative or toxic compounds, etc.). Alternatively, cells can be grown in transparent plates and read or transferred to microfuge tubes for centrifugation. The range of cell concentrations will need to be optimized to ensure the best dynamic range (see Standardization for further details).

2. Centrifuge at 250 x g for 5 minutes with a centrifuge equipped to handle microplates. Alternatively, transfer the cells to microfuge tubes for centrifugation and return to the plate to read.

3. Carefully discard the media supernate 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 supernate, 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 (see Reagent Preparation).

7. Incubate for 30 minutes at 37° C under CO₂ (or normal growth conditions).

8. Record fluorescence using a 490 nm excitation filter and a 520 emission filter. The fluorescence intensity is proportional to the number of viable cells (see Figure 1).

ADHERENT CELLS

1. Seed cells at varying densities (1,000 - 500,000 cells per mL) in appropriate medium in black-walled microplates and treat according to experimental protocol (varying amounts of proliferative or toxic compounds, etc.). Transparent plates may also be used to ensure cell adherence but background fluorescence may reduce assay sensitivity (see manufacturer’s recommendations for your fluorimeter). The optimal range of cell number may need to be optimized to ensure the best dynamic range (see Standardization for further details).

2. Discard the media supplement 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 (see Reagent Preparation).

5. Incubate for 30 minutes at 37° C under CO₂ (or normal culture conditions).

6. Record fluorescence using a 490 nm excitation filter and a 520 emission filter. The fluorescence intensity is proportional to the number of viable cells (see Figure 1).
SAMPLE EXPERIMENTAL RESULTS

Figure 1: 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 incubated with 1 μM Calcein AM for 30 minutes at 37° C under 5% CO2. Fluorescence values were obtained using a 485 nm excitation filter and a 520 nm emission filter in a BMG Laboratories’ FLUOstar OPTIMA Fluorimeter with a gain setting of 1600.

STANDARDIZATION

There are two measurement options; 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 absorbance relative to an experimental control.

To calibrate using cell number, determine the cell number in a sample and plate out dilutions in triplicate covering a range of 1,000 - 500,000 cells per mL in 50 μL of medium. Perform the standard assay. Determine averages of triplicate values and plot data as cell number per well versus fluorescence intensity.

To calibrate fluorescence values across microplates, the same gain setting must be used. Refer to the manufacturer’s instructions for your fluorometer.
<table>
<thead>
<tr>
<th>Problem</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low fluorescence values</td>
<td>Increase the concentration of Calcein AM used.</td>
</tr>
<tr>
<td></td>
<td>Check the health of the cells during incubation with Calcein AM (using Trypan Blue, etc).</td>
</tr>
<tr>
<td></td>
<td>Incubate the plate in the dark.</td>
</tr>
<tr>
<td>Poor triplicates</td>
<td>Ensure no bubbles are present in wells.</td>
</tr>
<tr>
<td></td>
<td>Pipette cells accurately.</td>
</tr>
<tr>
<td></td>
<td>Check the accuracy of the pipette.</td>
</tr>
<tr>
<td></td>
<td>Ensure that there is no loss of cells during wash steps.</td>
</tr>
<tr>
<td>High background</td>
<td>Use black-walled microplates.</td>
</tr>
<tr>
<td></td>
<td>Use Calcein AM DW Buffer.</td>
</tr>
<tr>
<td></td>
<td>Use freshly diluted Calcein AM.</td>
</tr>
<tr>
<td></td>
<td>Increase the washes to ensure media removal.</td>
</tr>
<tr>
<td></td>
<td>Shorten the incubation time with Calcein AM.</td>
</tr>
<tr>
<td></td>
<td>Decrease the number of cells per well.</td>
</tr>
</tbody>
</table>
REFERENCES