SensoLyte® Calcein Cell Viability Assay Kit *Fluorimetric*

Revision Number: 1.1	*Last updated: October 2014*
Catalog #	**AS-72126**
Kit Size	1000 Assays (96-well plate)

- ***Optimized Performance:*** This kit is optimized for detecting cell viability
- ***Enhanced Value:*** It provides ample reagents to perform 1000 assays in a 96-well format.
- ***High Speed:*** The cells can be stained and quantified within 2 hours.
- ***Assured Reliability:*** Detailed protocol and references are provided.

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	Calcein AM in anhydrous DMSO solution	0.5 mM, 100 µL
Component B	10X Assay buffer	100 mL

Other Materials Required (but not provided)

- 96-well or 384-well microplate: Black tissue culture microplate with clear bottom
- Fluorescence microplate reader: Capable of detecting excitation at 490±20 nm with emission at 520 ±20 nm

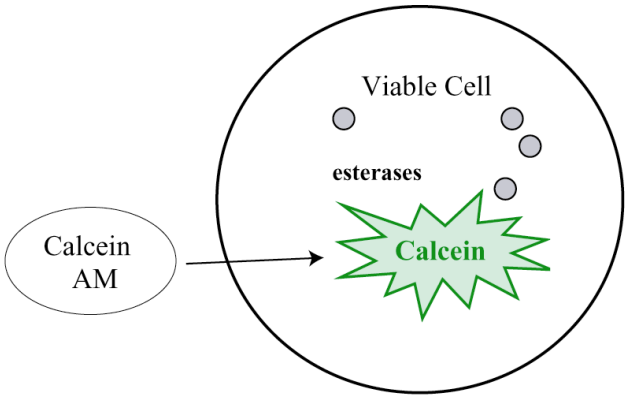
Storage and Handling

- Store Component A at –20^{0}C.
- Protect Component A from light and moisture.
- Component B can be stored at room temperature for convenience.

Introduction

Calcein acetoxymethyl ester (Calcein AM), a non-fluorescent, cell permeable compound, converts to fluorescent calcein when hydrolyzed by intracellular esterases in live cells.[1-2] The intensity of calcein fluorescence is proportional to the amount of live cells. Calcein is a photostable reagent. It is well retained in cells and is not influenced by intracellular pH. The bright green fluorescence of calcein can be monitored at Ex/Em=494nm /520 nm.

The SensoLyte® Calcein Cell Viability Assay Kit provides a rapid and sensitive assay to detect viable cells. Calcein AM does not affect cell proliferation or viability.[2-3] Calcein AM is used in cell invasion, adhesion, migration and other cell-based assays.



Scheme 1. Esterases in the living cells hydrolyze non-fluorescent Calcein AM to green fluorescent calcein.

Protocol

Note: Warm all kit components to room temperature before starting the experiment.

1. **Prepare cells.**

1.1 Seed 2x10[2-4] cells per well in a 96-well microplate. After adding the test compounds, incubate cells at 37°C for the desired time period.

Note: The seeding cell density may need optimization for each cell line.

1.2 Simultaneously establish the following control wells, as deemed necessary:

- Positive control contains the live untreated cells.
- Negative control contains culture medium without cells.
- Vehicle control contains cells and the vehicle used to deliver test compounds.
- Test compound control contains culture medium and test compound without cells.

2. **Prepare working solutions.**

2.1 1X Assay buffer: Dilute 10X assay buffer (Component B) to 1X in deionized water.

2.2 Prepare Calcein AM working solution: Dilute the Calcein AM stock solution (Component A) 1:1000 with 1X assay buffer. Prepare fresh working solution for each experiment.

3. **Perform the assay.**

3.1 Discard the culture medium from the wells and wash them twice with 200 µl of 1X assay buffer.

Note 1: Thoroughly remove the medium since phenol red and serum interfere with assay sensitivity.

Note 2: If using suspension cell line, centrifuge the plate to remove growth medium and wash cells (1000xg for 5 min). Suspension cells may also be washed in eppendorf tubes and then transferred back to the plate for reading.

3.2 Add 100 µl of Calcein AM working solution to each well.

3.3 Incubate the plate at 37^0C for 30 min or longer if necessary.

3.4 Measure fluorescence intensity at Ex/Em=490 nm/520 nm

4. **Data analysis.**

4.1 The fluorescence reading from the negative control well is the background fluorescence. This background reading has to be subtracted from the readings of the other wells to get the relative fluorescence unit (RFU).

4.2 The fluorescence reading in each well is an indication of the cell number in that well.

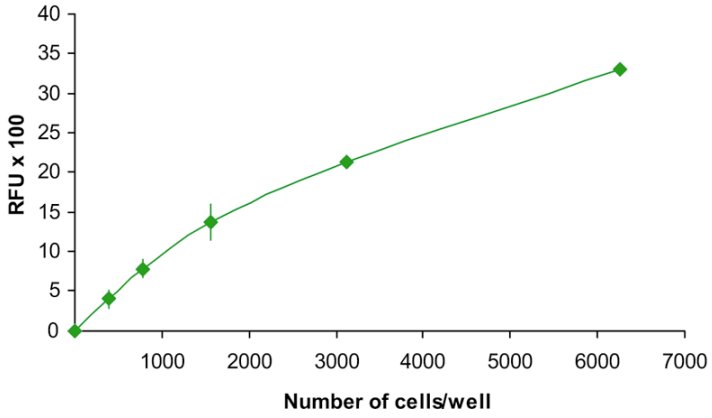


Fig1. 3T3 cells were seeded into a 96-well plate at the indicated densities. The following day, cells were washed with 1X assay buffer and incubated with Calcein AM for 1 hour. Fluorescence was recorded at Ex/Em=490 nm/ 520 nm (Flexstation 384II, Molecular Devices).

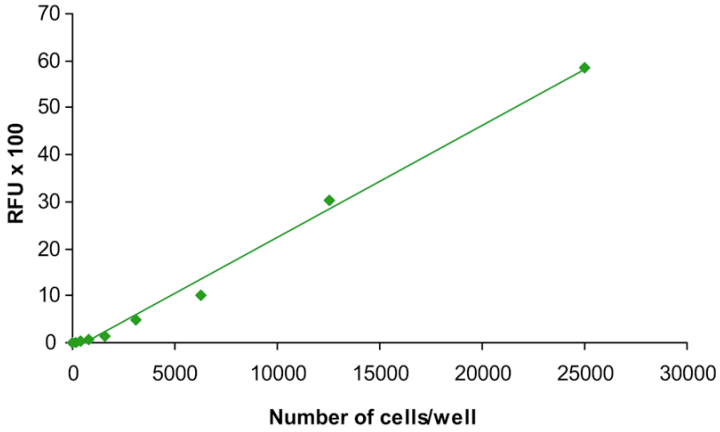


Fig 2. Fluorescent signal increase correlates with an increase of cell number. Jurkat cells were seeded into a 96-well plate at the indicated densities and incubated with Calcein AM diluted in 1X assay buffer for 1 hour. Fluorescence was recorded at Ex/Em=490 nm/ 520 nm (Flexstation 384II, Molecular Devices).

References

1. Braut-Boucher, F. et al. *J. Immunol. Methods* **178**, 41 (1995).
2. Akeson, AL. et al. *J. Immunol. Methods* **163**, 181 (1993).
3. Weston , SA. et al. *J. Immunol. Methods* **133**, 87 (1990).