FT-466251

Calceins

The best probe for cell viability and cell adhesion due to its greater retention in cells. It has been used as a neutral substrate for multidrug resistance protein.

Products Information

Product name cat.number	MW ($g \cdot mol^{-1}$)	$\lambda_{exc}/\lambda_{em}$ (nm)	mol. abs. ($M^{-1}cm^{-1}$)	Solubility	Comments
Calcein FP-466251, 100 mg	622.54	494 / 517	75 000	**DMSO, DMF, CH_3CN, $CHCl_3$ and EtOAc**	Highly negatively charged, thus retained in the cytoplasm.
Calcein, AM FP-895514, 1 mg FP-895515, 20x50 µg	994.88	<300/⦸ before hydrolysis 494 / 517	75 000 after hydrolysis		Becomes fluorescent upon hydrolysis. Membrane-permeant dye introduced into cells via incubation. Once inside the cells, calcein AM is hydrolyzed by endogenous esterase into the calcein.
Calcein, AM, 1 mg/ml in DMSO FP-855422, 1 ml					
Calcein, AM, 4 mM in DMSO FP-FI9820, 100 µl					
Calcein AM, Orange FP-ZE7840, 1 mg	880	525 / 550			

Calcein free acid: Bis[N,N-bis(carboxymethyl)aminomethyl]fluorescein
Calcein AM: 3',6'-Di(O-acetyl)-2',7'-bis[N,N-bis(carboxymethyl)aminomethyl]fluoresceintetraacetoxymethyl ester

Storage: **Indicator salts** can be stored at +4°C$_{(K)}$ desicated and protected from light.
AM esters can be stored desicated and protected from light at –20°C$_{(M)}$.

Introduction

Calcein dye is a polyanionic derivate of fluorescein that exhibits fluorescence that is essentially independent of pH between 6.5 and 12. It is well retained in cells. These features have made it a popular and versatile dye for various applications, including cell volume changes in neurons and other cells, endocytosis, gap junctional communication, membrane integrity and permeability, angiography, liposomes…
It is worthy to notice that calcein fluorescence is decreased at low pH values, and it is strongly quenched by several ions, including Fe^{3+}, Co^{2+}, Cu^{2+} and Mn^{2+} at physiological pH (not by Ca^{2+} or Mg^{2+} ions). PH and Ions levels should thus be monitored.

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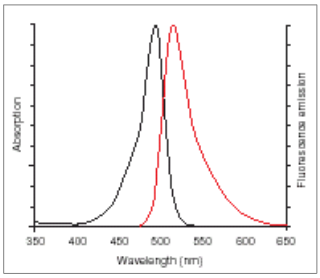

Fluorescence of calcein at pH9.0

Free acid and salt form are membrane-impermeant, but can be introduced into cells via microinjection.

AM ester is membrane-permeant and enters readily cell membranes. Intracellular esterases convert it into calcein. The DMSO solution is more conveninent (time saving, reduce solubilization variability) especially for more reproductible screening assays.

Directions for use

Handling and Storage

Free acid and salt form of calcein are soluble in DMSO, DMF and is slightly water soluble (pH>6).
AM form of calcein is susceptible to hydrolysis. It should be dissolved in DMSO .It should be prepared immediately before use and should be used with 12 hours, preferably within 3 to 4 hours.

Guidelines for use – for microscopy studies

The following procedure is found suitable for NIH 3T3, PtK2 and MDCK cells. For the other cell types, the exact dyes concentration and incubation time will vary somewhat. For example, cells that have higher esterase activity may need a lower calcein AM concentration.
A conventional fluorescein long-pass filter may be used. This is particularly a good choice if a red fluorescent dye is also used in the experiment. Alternatively, a standard fluorescein band-pass filter can be used for viewing calcein fluorescence.

1- Adherent cells are cultured as usual and then should be washed prior to the assay with 500-1000 volumes of a phosphate buffer such as Dubelcco's PBS (KCl (200mg/ml), KH_2PO_4 (200mg/ml), NaCl (8g/l) and Na_2HPO_4(1.15g/l) to remove any serum esterase activity that may be present in the growth media.

Note: esterase activity in the media will hydrolyse calcein AM and generate background fluorescence. Similarly, non-adherent cells are washed in a test tube with 500-1000 volumes of D-PBS, followed by centrifugation to sediment.

2- Add 5µl of 4mM calcein AM in DMSO (warm to room temperature) to 10ml of sterile, tissue culture grade D-PBS. Vortex to give 2 µM calcein AM working solution.

3- Add 100-150µl of the above working solution to the coverslip containing the cells. Cover the coverslip with a petri dish to prevent contamination or evaporation of the solvent, and then incubate the cells for 30 to 45 minutes at room temperature. The exact incubation time vary, depending on the dye concentration and temperature. Higher temperature or higher dye concentration will require less incubation time.

Note: the working solution shoul cover all the cells.

4- Add small drop (10µl) of D-PBS to a clean microscope slide.

Note: carefully invert the coverslip containing the stained cells and mount it on the microscope slide. The coverslip may be sealed to avoid drying.

5- View the stained cells.

Guidelines for use – for fluorescence microplate

The filters for calcein are:
Excitation: 485 +/-10nm (fluorescein filter)
Emission: 530+/-12.5nm

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The following procedure is found suitable for mouse leukocytes. For other cell types the exact dye concentration and incubation time will vary somewhat. In general, one should use the lowest dye concentration that gives sufficient fluorescence signal.

1- Culture adherent cells directly in the multiwell plate as usual for 2 to 3 days, followed by washing the cells with 500-1000 volumes of D-PBS to remove any serum esterase activity that could contribute to background fluorescence.

2- Wash non adherent cell in a test tube also with 500-1000 volumes of D-PBS, followed by centrifugation to sediment the cells.

3- Add enough of the cells in a buffer to the wells so that the well bottoms are covered. For example, 100 µl of the cell containing buffer is sufficient for round-bottomed wells. Use an appropriate amount of the cell containing buffer for other types of wells.

4- Add 5 µl of 4mM calcein AM in DMSO (warm to room temperature) to 10ml of sterile, tissue culture grade D-PBS. Vortexed to give 2 µM calcein AM working solution.

5- Add 100 µl of the cell-containing buffer to each well, followed by addition of 100 µl of the 2 µM calcein AM solution to obtain a working concentration of 1µM.

6- Incubate the cells for 30 to 45 minutes at room temperature. The exact incubation time vary, depending on the dye concentration and temperature. Higher temperature or higher dye concentration will require less incubation time.

7- Measure the fluorescence using the recommended optical filters.

Guidelines for use – for flow cytometry

The protocol used for fluorescence microscopy can be adapted for flow cytometry.

Guidelines for use – Ca+/Mg+/Metalc ions dosage in solution

Calcein is used to quantitate Ca+ in solution. The sample (mineral water) is mixed with Calcein, that can be assayed by fluorescence. A colorimetric assay combines Calcein with NET/EDTA method (EDTA chelate displaces Ca+ and Mg+ ions form binding to Calcein and a colored complexion of Eriochrome T). This method has even been adapted to accomadate the presence of Fe, Al, Ti [(Zalessky 1960)].

Reference:

Z. Zalessky; Analytica Chimica Acta, Vol.23, 1960, Pages 523-530; Dosage direct de calcium et de magnésium en présence de fer, aluminium et titane par le sel disodique de l'acide éthylènediaminetétracétique (edta); Abstract

Calcein fluoresces in the presence of certain metal cations such as Al (III), Ba (II), Cu (II), Mg (II), Hg (II) and Zn (II) under basic conditions. Therefore, calcein can be used for direct fluorimetric titration of these heavy metal ions as well as Ca (II).

Calcein self-quenches at concentrations above 100 mM, that allows also other applications

References

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Related products

- Calcein FluoProbesPure grade FP-HG6257
- Annexin V-FluoProbes 488, FP-BH9390
- Ethidium Homodimer I, FP-25810A
- Ethidium Homodimer III, FP-BP9340
- Hoechst 33342 (and others), FP-59046A
- Dihydroethidium, FP-52492A
- Cyclosporin A, >99% Multidrug Resistance Assay, FP-C71434
- Other calceins: blue, Violet405, Violet500, Orange, red

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