Calcein AM

Table 1 - Materials Needed	Most Items Available from ION Biosciences	
Name	**Volume**	**Description/Purpose**
Calcein AM - Available in Two Forms: ⇒ Dry - 50 µg or 1 mg Vials ⇒ Solution - 2 mM in DMSO	 1 x 50 µg Vial 10 µL	Membrane Permeable Dye Indicator
DMSO[1]	25 µL	Solvent for Dissolution of Dye
50X TRS (optional)[2]	200 µL	Extracellular Fluorescence Masking Agent
Assay Buffer - We Suggest One of the Following: ⇒ 1X HEPES-Buffered Hank's Balanced Salt Solution ⇒ 1X Phosphate Buffered Saline	 10 mL 10 mL	Buffer for Preparing Dye Loading Solutions

Description

Calcein AM is a membrane-permeant, non-fluorescent form of Calcein that enters cells passively. Once inside the cytosol of cells, Calcein AM is converted to green-fluorescent Calcein by ubiquitous esterases in viable cells, resulting in uniform cytosolic fluorescence (Ex/Em 495 nm/515 nm). Calcein, a polyanionic dye, is membrane-impermeant and is well retained within the cytosol of healthy cells with intact cell membranes.

Laboratory Procedures - General Considerations

1. Before you begin, make sure that you have all the additional reagents and materials you will need for the successful completion of your experiment if conducting additional assays with Calcein AM.
2. In addition to reagents, a microscope and fluorescence plate reader that is capable of providing an excitation source at ~ 490 nm and measuring emission at ~530 nm is required.
3. Optimal dye concentrations will vary depending on cell type and application. Recommended dye concentrations range between 0.1 µM and 10 µM.
4. Aqueous solutions of Calcein AM are susceptible to hydrolysis; therefore, all dye load solutions should be used as quickly as possible and no later than 24 hours after preparation.
5. Serum-containing preparations will increase extracellular Calcein fluorescence. When possible, a wash step can remove extracellular fluorescence. Optionally, TRS can be added in some applications to mask extracellular fluorescence when a no-wash procedure is preferred.
6. Calcein cannot withstand fixation after staining.

Plate Reader

1. Seed cells in a 96-well (or 384-well) plate and treat with test compounds of your choosing prior to staining.
2. Remove Calcein AM from freezer and allow the dye to warm to room temperature.
3. Add 25 µL of DMSO[1] to the dry Calcein AM dye tube and vortex to mix completely. Proceed directly to **step 4** if using Calcein AM, 2 mM in DMSO solution.
4. Prepare a **dye loading solution** that contains 2 µM Calcein AM (1:1000 dilution) in either 1X HEPES-buffered Hank's Balanced Salt Solution (HHBSS) or Phosphate-buffered Saline (PBS) or other serum-free medium or buffer. For example, add 10 µL of Calcein AM to 10 mL of 1X HHBSS. Vortex briefly to mix.
5. Optional: Wash the cells with serum-free buffer or medium to remove serum. For suspension cells, use a centrifuge to pellet cells, then resuspend in 100 µL of serum free medium or buffer. The wash solution can be aspirated from wells prior to the addition of **dye loading solution** if desired.
6. Optional: Add 200 µL of 50X TRS[2] to the **dye loading solution**. Adjust 1X HHBSS volume in **step 4** to 9.8 mL. TRS minimizes extracellular fluorescence and is recommended when long incubation times with **dye loading solution** or serum containing media are used.
7. Add **dye-loading solution** prepared in **step 4** directly to cells. We recommend 100 µL/well for a 96-well plate.
8. Incubate cells for 30 - 45 min at room temperature or 37°C. Protect from light.
9. Measure fluorescence using a microplate reader. Use Ex/Em ~495 nm/515 nm or FITC settings. See **Table 1** for recommended instrument settings.

Microscopy Assay

1. Remove Calcein AM from freezer and allow the dye to warm to room temperature.
2. Add 25 µL of DMSO[1] to the dry Calcein AM dye tube and vortex to mix completely. Proceed directly to **step 3** if using Calcein AM, 2 mM in DMSO solution.
3. Prepare a **dye loading solution** that contains 2 µM Calcein AM (1:1000 dilution) in either 1X HEPES-buffered Hank's Balanced Salt Solution (HHBSS) or Phosphate-buffered Saline (PBS) or other serum-free medium or buffer. For example, add 10 µL of Calcein AM to 10 mL of 1X HHBSS. Vortex briefly to mix.
4. Optional: Wash the cells with serum-free buffer or medium to remove serum.
4. Add sufficient volume of the **dye loading solution** prepared in **step 3** to completely cover cells.
5. Incubate cells for 30 - 45 min at room temperature or 37°C. Protect from light.
6. Optional: Replace **dye loading solution** with fresh buffer or medium prior to imaging.
7. Image stained cells using fluorescence microscopy using GFP or FITC filters.

Flow Cytometry Assay

1. Remove Calcein AM from freezer and allow the dye to warm to room temperature.
2. Add 25 µL of DMSO[1] to the dry Calcein AM dye tube and vortex to mix completely. Proceed directly to **step 3** if using Calcein AM, 2 mM in DMSO solution.
3. Prepare a **dye loading solution** that contains 2 µM Calcein AM (1:1000 dilution) in either 1X HEPES-buffered Hank's Balanced Salt Solution (HHBSS) or Phosphate-buffered Saline (PBS) or other serum-free medium or buffer. For example, add 10 µL of Calcein AM to 10 mL of 1X HHBSS. Vortex briefly to mix.
4. Pellet cells via centrifugation, remove supernatant, then resuspend in 100 µL of serum free medium or buffer.
5. Add 100 µL of **dye loading solution** prepared in **step 3** directly to cells.
6. Incubate cells for 30 - 45 min at room temperature or 37°C. Protect from light.
7. Pellet cells again via centrifugation and resuspend in preferred flow cytometry buffer.
8. Analyze cells using a flow cytometer. To detect calcein (+) cells, use FITC settings.

Table 1	**Recommended Instrument Settings**
Setting	**Recommendation**
Read Mode (Plate Readers)	'Bottom' read mode only
Ex/Em wavelengths[3]	490 nm /530 nm
Cutoff wavelength	515 nm
Filter selection	GFP or FITC
Contact support@ionbiosciences.com for additional recommendations and guidance on optimizing to your application.	

Laboratory Procedures - Footnotes

[1] DMSO is hygroscopic and should be stored tightly closed. Wet solvent causes difficulties with dissolution of the dye.

[2] TRS is a membrane impermeant dye useful for masking extracellular fluorescence. Caution is advised when using TRS or any other extracellular masking solutions as they may have undesirable effects on assay performance for the target of interest.

[3] To prevent bleed-through or spectral overlap, the Ex/Em wavelengths may need to be optimized by broadening the interval between the wavelengths.

Additional Information

Calcein AM, 2 mM in DMSO must be shipped frozen.

Additional dye indicator and buffer reagents can be purchased either directly from our website or by contacting our Sales Department.

Table 2 Available Dye Indicators	Available Sizes	
Name	**Size**	**Catalog #**
Calcein AM	1 mg x 1 Vial 50 µg x 20 Vials	1071B 1071E
Calcein AM, 2 mM in DMSO	0.5 mL	5030
50X TRS	20 mL	7060A
1X HEPES-buffered Hanks Balanced Salt Solution (HHBSS)	100 mL	7001