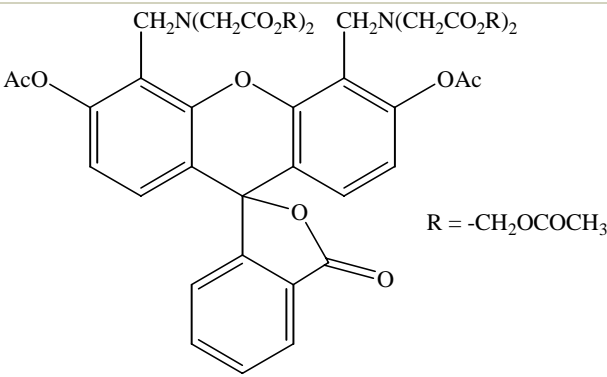


Calcein AM Solution (1 mg/ml)

3',6'-Di(O-acetyl)-2',7'-bis[N,N-bis(carboxymethyl)aminomethyl]
fluorescein tetraacetoxymethyl ester

Instruction Manual

Catalog Number	PK-CA707-80011-2
Description	Calcein-AM is a widely used membrane-permeant cell marker that readily passes through the cell membrane of viable cells because of its enhanced hydrophobicity as compared to Calcein. After the non-fluorescent Calcein-AM permeates into the cytoplasm, it is hydrolyzed by endogenous esterases into the highly negatively charged green fluorescent Calcein, which is retained in the cytoplasm. Among other reagents, including BCECF-AM and Carboxy-fluorescein diacetate, Calcein-AM is the most suitable fluorescent probe for staining viable cells because of its low cytotoxicity. Calcein does not inhibit any cellular functions such as proliferation or chemotaxis of lymphocyte. In addition, viability assays using Calcein are reliable and correlate well with the standard ^{51}Cr -release assay. Calcein AM is an excellent tool for the studies of cell membrane integrity and for long term cell tracing. The excitation and emission wavelengths of calcein are 490 nm and 515 nm, respectively.
Quantity	1 mg/ml in anhydrous DMSO
Excitation / Emission Maxima	$\lambda_{\text{abs}} < 300\text{nm}$ (before hydrolysis); $\lambda_{\text{abs}}/\lambda_{\text{em}} = 494/517\text{ nm}$ (pH 9, after hydrolysis). - (100 μl /10 ml 1 M NaOH): >0.77 (500 nm) - (100 μl /10 ml DMSO): <0.010 (500 nm)
Molecular Structure	 <p>R = $-\text{CH}_2\text{OCOCH}_3$</p>
Molecular Weight / Molecular Formula	994.86 kDa; $\text{C}_{46}\text{H}_{46}\text{N}_2\text{O}_{23}$
Purity	$>90\%$ (as determined by TLC)
Appearance / Formulation / Solubility	Off-white liquid.
Storage & Stability	Store desiccated at -20°C upon receiving. Protect from light, especially when in solution.
Intended Use	For in vitro research use only. Not for diagnostic or therapeutic procedures.
Applications	<p>General Staining Procedure:</p> <ol style="list-style-type: none">1. Prepare 1 mM Calcein-AM solution with DMSO and dilute to prepare 1-50 μM Calcein-AM solution with PBS.^{a)}2. Add Calcein-AM solution with 1/10 of the volume of cell culture medium to the cell culture.^{b)}3. Incubate the cell at 37°C for 15-30 minutes.4. Wash cells twice with PBS or an appropriate buffer.5. Observe the cells using a fluorescence microscope with 490 nm excitation and 515 nm emission filters. <p>a) If the Calcein-AM loading into cells is difficult, use a detergent such as Pluronic F127. b) Or you may replace the culture medium with 1/10 concentration of Calcein-AM buffer solution.</p>

References	<p>References:</p> <p>1) J. Immunol. Meth. 177, 101(1994)</p> <p>2) J. Immunol. Meth. 172, 227(1994)</p> <p>3) J. Immunol. Meth. 172, 115(1994)</p> <p>4) Human Immunol. 37, 264(1993)</p>
Caution	<p>Potentially harmful. Avoid prolonged or repeated exposure. Avoid getting in eyes, on skin, or on clothing. Wash thoroughly after handling. If eye or skin contact occurs, wash affected areas with plenty of water for 15 minutes and seek medical advice. In case of inhaling or swallowing, move individual to fresh air and seek medical advice immediately.</p>

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