Fluorometric Cell Viability Kit II (Calcein)

PromoKine

Instruction Manual

Product Name	Product Description	Size	Catalog Number
Fluorometric Cell Viability Kit II (Calcein)	Fluorometric Cell Viability Kit II (Calcein)	1000 assays	PK-CA707-30026

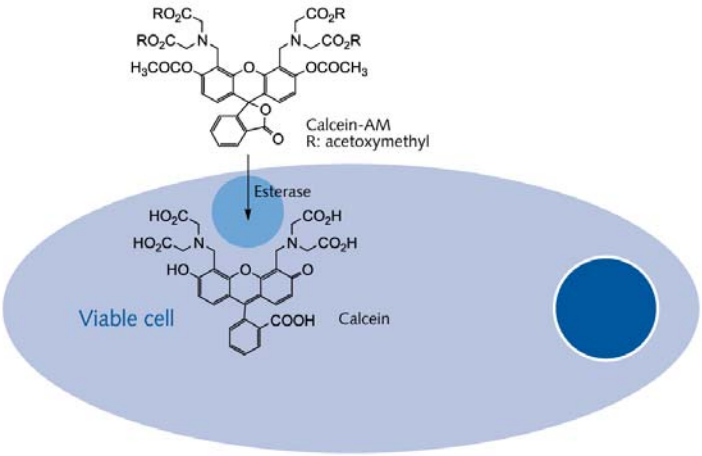
Introduction

Calcein AM is a widely used green fluorescent cell marker. Calcein AM is membrane-permeant and thus can be introduced into cells via incubation. Once inside the cells, calcein AM, a nonfluorescent molecule itself, is hydrolyzed by endogenous esterases into the highly negatively charged green fluorescent calcein. The fluorescent calcein is membrane-impermeant and thus retained in the cytoplasm in live cells. Calcein AM has been served as an excellent tool for the studies of cell membrane integrity and for long-term cell tracing. The Fluorometric Cell Viability Kit II (Calcein) has been designed to quantify live cell numbers based on the presence of their cytoplasmic membrane integrity. It is a true end-point assay for cell viability. The fluorescent signal is monitored using 485 nm excitation wavelength and 530 nm emission wavelength (Ex/Em Calcein: 494/517 at pH 8.0). The fluorescent signal generated from the assay is proportional to the number of living cells in the sample.

Kit Contents

Calcein AM (2 mM in anhydrous DMSO): 100 µl

Assay mechanism



Storage and Stability

Upon receipt, the kit should be stored at -20°C and protected from light. Avoid exposure to moisture. Stored properly, the kit components should remain stable for 6 months. Note: It is important to protect product from moisture to prevent hydrolysis of the Calcein AM during storage. If you aliquot the Calcein AM stock solution, store the vials in a desiccator, or inside an airtight secondary container containing desiccant.

Experimental Protocol

Preparation of Calcein AM working solution

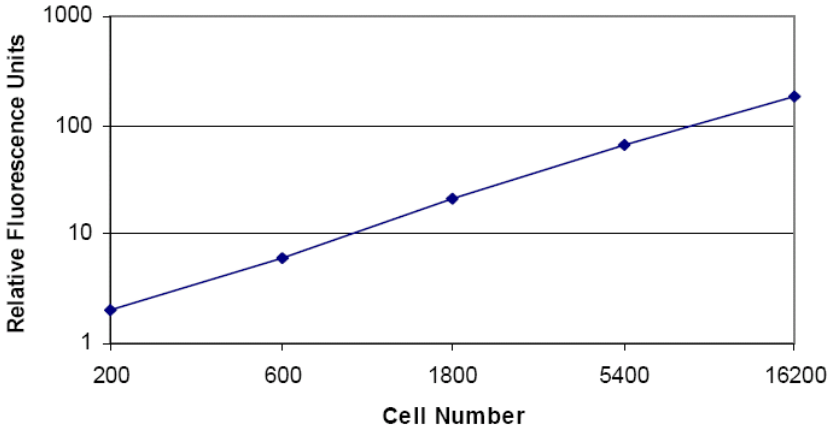
Note: Aqueous solutions of Calcein AM are susceptible to hydrolysis. Working solution should be used within one day.

1. Remove the Calcein AM reagent stock solution from the freezer and allow to warm up to room temperature for 30 minutes.
2. Add 10 µl of the supplied 2 mM Calcein AM stock solution to 10 ml of PBS, vortexing to ensure thorough mixing. This gives an approximately 2 µM Calcein AM working solution.

Note: the optimal concentration of Calcein AM may vary depending on cell type. In general it is best to use the lowest dye concentration that gives sufficient signal. The range of titration is within 0.1 to 10 µM for Calcein AM. The standard 2 µM Calcein AM working solution is suitable for NIH3T3, PtK2, HeLa and MDCK.

Calcein AM Cell Viability Assay

3. Plate cells into 96-well tissue culture plates. Black walled plates are recommended for fluorescence-based assays. For adherent cells, plate cells at least one day before the assay. Include wells without cells as a background control
4. Note: you may wish to plate a titration curve of cell density to determine the linear range and optimal seeding density for your assay and cell type.
5. Carry out any experiment cell treatments.
6. Aspirate medium from each well of the plate.
7. Note: serum in cell culture medium may contain esterase activity, which can increase background fluorescence. Cells can be rinsed in PBS at this step to reduce background caused by residual serum.
8. Add 100 µl 2 µM Calcein AM in PBS to each well. Incubate at 37°C for 30 minutes or longer.
9. Measure the fluorescence on fluorescence plate reader with the excitation wavelength at 485 nm and the emission wavelength of 530 nm.



Quantitation of Hela cells using PromoKine's Cell Viability Kit II (Calcein). Cells were counted, diluted to the indicated cell numbers, and plated into the designated wells of a 96-well plate one day before the assay. On the next day, 100 µl Calcein AM solution was added into each well after removal of growth medium. After 30 min incubation at 37°C, fluorescence signal was detected using SpectraMAX GerminiXS fluorescence plate reader (Molecular Device).

Intended Use

For in vitro research use only. Not for diagnostic or therapeutic procedures.

References

1. Papadopoulos NG, Dedoussis GV, Spanakos G, Gritzapis AD, Baxevanis CN, Papamichail M. An improved fluorescence assay for the determination of lymphocyte-mediated cytotoxicity using flow cytometry. J Immunol Methods 177, 101 (1994).
2. De Clerck LS, Bridts CH, Mertens AM, Moens MM, Stevens WJ. Use of fluorescent dyes in the determination of adherence of human leucocytes to endothelial cells and the effect of fluorochromes on cellular function. J Immunol Methods. 1994 Jun 3; 172(1):115-24.
3. Wang XM, Terasaki PI, Rankin GW Jr, Chia D, Zhong HP, Hardy S. A new microcellular cytotoxicity test based on calcein AM release. Hum Immunol 37, 264 (1993).

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