Calcein AM Cell Viability Assay Kit

Catalog Number: 30026 (1000 assays)

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Description

Calcein AM is a widely used green fluorescent cell marker. Calcein AM is membrane-permeant and can be introduced into cells via incubation. Once inside the cells, Calcein AM (a nonfluorescent molecule) is hydrolyzed by endogeous esterase into the highly negatively charged green fluorescent calcein. The fluorescent calcein is 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 due to its lack of cellular toxicity [1,2]. It has also been used for quantifying cells number [1-3]. Calcein AM Cell Viability Assay Kit is 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. The fluorescent signal generated from the assay is proportional to the number of living cells in the sample.

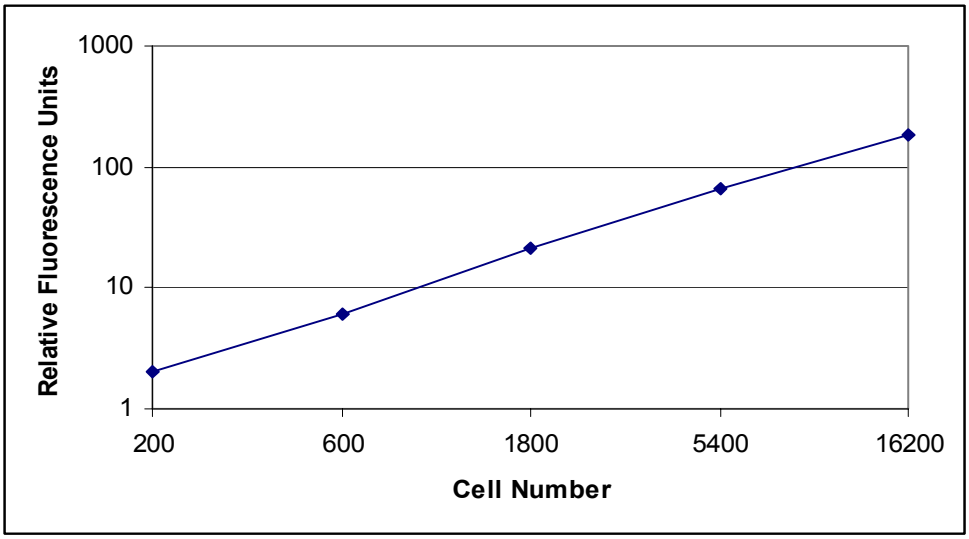

Figure 1. Quantitation of Hela cells using Biotium's Calcein AM Cell Viability Assay Kit. Cells were counted, diluted to the indicated cell numbers, and plated into the designated wells of a 96-well plate one day before the assay. On the next day, 100µL Calcein AM solution was added into each well after removal of growth medium. After 30 min incubation at 37°C, fluorescence signal was detected using SpectraMAX GerminiXS fluorescence plate reader (Molecular Device).

Kit Component

1 vial of 100µL 2mM Calcein AM in anhydrous DMSO.

Storage and Handling

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.

Experimental Protocol

Preparation of Calcein AM working solution

1.1 Remove the Calcein AM reagent stock solution from the freezer and allow to warm up to room temperature for 30 min.

1.2 Add 10µL of the supplied 2mM Calcein AM stock solution to 10mL of PBS, vortexing to ensure thorough mixing. This gives an approximately 2µM Calcein AM working solution.

Note: 1) aqueous solutions of Calcein AM are susceptible to hydrolysis (see *Storage and Handling of Reagents*). Aqueous working solutions should therefore be used within one day.

2) The optimal concentrations are likely to vary depending on the 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 10μM for Calcein AM. The standard Calcein AM working solution is suitable for NIH3T3, PtK2, Hela and MDCK.

Calcein AM Cell Viability Assay

1. Plate cells into 96-well tissue culture plates a day before the assay. In general, cells should be seeded at densities around 5000 cells per well for adherent cells.
2. Carry out your experiment by adding chemicals or biological agents into appropriate well and incubate with cells for a certain period of time.
3. Aspirate medium from each well of the plate.
4. Add 100μL 2μM Calcein AM in PBS to each well.
5. Incubate at 37°C for 30 min or longer.
6. Measure the fluorescence on fluorescence plate reader with the excitation wavelength at 485 nm and the emission wavelength of 530 nm.

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).