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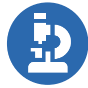
Diagnostik & molekulare Diagnostik

 Laborgeräte & Service

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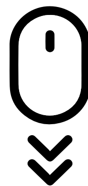

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
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- Gefahrgutzuschlag
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Product Information

Calcein AM Cell Viability Assay

Catalog Number: 30026

Unit Size: 1000 assays (96-well plate format)

Contents
100 uL 2 mM Calcein AM in anhydrous DMSO

Storage and Handling
Upon receipt, store at -20°C, desiccated and protected from light. Product is stable for at least 6 months from date of receipt when stored as recommended. 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.

Spectral Properties
Ex/Em (calcein): 494/517 nm (pH 8)

Product Description

Calcein AM is a widely used green fluorescent cell marker. Calcein AM is membrane-permeable and can be introduced into cells via incubation. Once inside the cells, non-fluorescent calcein AM is hydrolyzed by cellular esterases into the green fluorescent dye calcein. Calcein is highly negatively charged and is retained in the cytoplasm of healthy cells. Calcein AM has been used for studies of cell membrane integrity (1) and for long-term cell tracing due to its lack of cellular toxicity (2-3). It has also been used for quantifying viable cell numbers (2-4). The Calcein AM Cell Viability Assay is an end-point assay for cell viability to quantify live cell numbers. The fluorescent signal generated from the assay is proportional to the number of living cells in the sample (Figure 1).

References

1. 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).

2. Erik J. Suuronen, Christopher R. McLaughlin, Peter K. Stys, Masatsugu Nakamura, Rejean Munger and May Griffith, Functional Innervation in Tissue Engineered Models for InVitro Study and Testing Purposes Toxicological Sciences, 82, 525–533 (2004)

3. 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).

4. 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.

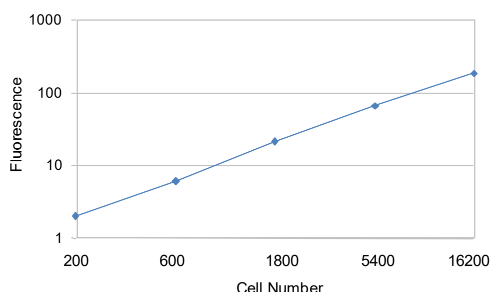

Figure 1. Quantitation of HeLa cell numbers using the Calcein AM Cell Viability Assay Kit. Cells were plated in 96-wells 24 hours before assay.

Assay 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 min.
2. Add 10 uL of the supplied 2 mM calcein AM stock solution to 10 mL of PBS, and vortex to mix. This gives 2 uM 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 uM for Calcein AM. The standard 2 uM Calcein AM working solution is suitable for NIH3T3, PtK2, HeLa and MDCK.

Cell viability assay

1. 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

   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.
2. Carry out any experiment cell treatments.
3. Aspirate medium from each well of the plate.

   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.
4. Add 100 uL 2uM 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.

Related Products

Catalog number	Product
30002	Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells
30025	Resazurin Cell Viability Assay Kit
30006	MTT Cell Viability Assay Kit
30007	XTT Cell Viability Assay Kit
30020	ATP-Glo™ Bioluminometric Cell Viability Assay Kit
30068	ViaFluor™405-SE Cell Proliferation Kit
30050	CFDA SE Cell Proliferation Assay Kit
30029	NucView™ 488 Caspase-3 Assay Kit for live cells
30065	Apoptosis & Necrosis Quantitation Kit Plus
30066	Apoptotic, Necrotic & Healthy Cells Quantitation Kit Plus

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