**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).

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

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**Preparation of calcein AM working solution**

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.
3. 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.

**References**


**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.

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