

Product Information

Calcein AM Cell Viability Assay

Catalog Number: C6003

Product Size: 100 µL Calcein AM, 2 mM in anhydrous DMSO (200T)

500 µL Calcein AM, 2 mM in anhydrous DMSO (1000T)

Application Scope: Cell tracing and tracking

Parameters

Ex/ Em: 494/517 nm (pH=8)

Storage

Store at -20°C and protect from light. When stored as directed, product is stable for at least 6 months.

Description

Calcein, AM is a kind of fluorescent labeling reagent for living cells. It can penetrate cell membrane and enter the cell, and be cutted by esterase inside the cell. Calcein is highly negatively charged and can be trapped in the cytoplasm of normal cells, giving off bright green fluorescence. Because of its low toxicity, Calcein, AM can be used to study cell membrane integrity and long-term cell tracing experiments. In addition, it can also be used for living cell quantification. The fluorescence signal is directly proportional to the number of living cells.(Fig.1)

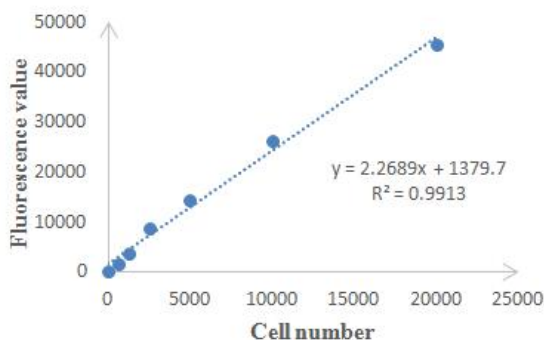


Fig.1 Number of HeLa cells. Cells were inoculated in 96 well plate 24 hours before detection.

Protocol

1. Configure working solution of Calcein, AM

1). Remove Calcein,AM storage solution from the frozen state, and allow it to stand for 30 minutes to recover to room temperature.

2) Add 10 µL 2 mM Calcein, AM storage solution into 10 mL PBS, mix well by vortex, and prepare into 2 µM Calcein, AM working solution.

Note: Recommended concentration of Calcein, AM working solution is 0.1-10 µM, and the optimal concentration depends on specific cell type. 2 µM of Calcein, AM working solution is suitable for the following types of cells: NIH3T3, PtK2, HeLa and MDCK.

2. Detection of cell viability

1). According to the experimental requirements, cells were inoculated on black 96 well culture plate. Adherent cells should be inoculate the day before detection, and the blank control well should be set, too.

2). Cells should be treated properly according to the experimental requirements.

3). Suck out culture solution of each well. Note: the serum in the medium may contain esterase activity, which will enhance the background fluorescence. Therefore, in this step, PBS can be used to clean cells to reduce the influence of residual serum.

4). Add 100 µL 2 µM Calcein, AM working solution into each well. Incubate at 37 °C for 30 min.





5). Fluorescence in the culture plate is measured by fluorescence detector with excitation wavelength of 485 nm and emission wavelength of 530 nm.

Note

Calcein, AM will decompose when encountering moisture. So please refrigerate in a closed manner at -20 °C to prevent moisture from entering.

