Calcein AM

Cat #: BMD0064 Size: 1 mg/10 mg

	Calcein AM		
REF	**Cat #:** BMD0064	LOT	**Lot #:** Refer to product label
	Application range: Nuclear staining reagents for DNA staining		**Recommended working concentrations:** 1-50 μM
	Excitation/Emission wavelengths: Ex/Em=494/517 nm (pH=8)		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

Calcein AM has the molecular formula $C_{46}H_{46}N_2O_{23}$, molecular weight 994.9, CAS number 148504-34-1. Calcein AM is a cell staining reagent that fluorescently labeled living cells. It can penetrate the cell membrane and enter the cell, where it is cleaved by intracellular esterase to form Calcein, thus being trapped in the cell and emitting strong green fluorescence. Compared with other similar reagents such as BCECF, AM and CFDA, Calcein AM showed very low cytotoxicity. The excitation and emission wavelengths of Calcein were 490 nm and 515 nm, respectively. Calcein AM stained only viable cells. It can be used in combination with PI to distinguish live cells from dead cells. Since the optimal staining conditions differ for different cell lines, we recommend that the optimal concentration of Calcein AM be determined according to the actual situation.

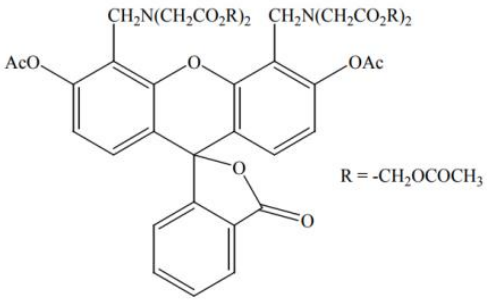

Figure 1. Molecular diagram

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
Calcein AM	1 mg	10 mg	-20℃, protected from light

Materials Required but Not Supplied

• Fluorescent microscope, precision pipettes, disposable pipette tips, PBS

Assay Procedure

1. 1 mM solution of Calcein AM was prepared with DMSO and diluted with PBS to make Calcein AM solutions ranging from 1 to 50 μM.

Note: The concentration of Calcein AM is different for different cells, and 2 μM Calcein AM is suitable for NIH3T3, PtK2, HeLa, MDCK, etc.

2. For staining adherent cells such as HeLa, the cells were first digested with Trypsin EDTA, etc. to prepare cell suspension.

3. The cell suspension was centrifuged for 3 min (1,000 rpm), the supernatant was removed, PBS buffer was added, the number of cells was adjusted to 10^5-10^6/mL, and then thoroughly mixed with a pipettor.

Note: Since serum and others in the medium contain esterase, which will break down Calcein AM and cause the blank background value to rise, it needs to be centrifuged several times and washed with PBS several times until it is completely washed.

4. 50 μL of diluted Calcein AM solution was added to 200 μL of cell suspension and incubated for 15 min at 37℃.

5. Drop an appropriate amount of stained cell solution onto the cover slip. Cells were observed by fluorescence microscopy with an excitation wavelength of 490 nm and an emission wavelength of 515 nm filter.

Note: If Calcein AM is difficult to enter cells, it can be treated with a surfactant such as Pluronic F127.

Precautions

1. Please immediately centrifugal the product to the bottom of the tube before use, and then conduct the subsequent experiments.

2. The ester bond of Calcein AM will decompress when it meets moisture. After use, please store it at -20℃ in the dark, sealed and frozen to prevent moisture from entering.

3. Please use the Calcein AM stock solution in time after dilution, and try to use it on the spot.

4. Fluorescent dyes all have quenching problems, please try to avoid light to slow down the quenching.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.