BCECF AM (pH Fluorescent probe，5mM)

B744102

Storage temperature: -20℃.Avoid freeze/thaw cycle. Store in the dark.

Introduction:

BCECF, AM is a fluorescent dye that can penetrate cell membranes to detect intracellular pH. BCECF, AM itself is non-fluorescent. Once inside the cell, it can be cleaved by intracellular esterases to form BCECF, which is then retained within the cell. BCECF can be excited to produce green fluorescence under appropriate pH conditions. The maximum excitation and emission wavelengths vary with pH, with the maximum excitation wavelength around 503 nm and the maximum emission wavelength around 520 nm. For practical detection, it is recommended to use an excitation wavelength of 488 nm and an emission wavelength of 535 nm. BCECF, AM is widely used not only in the study of mammalian cells but also for detecting intracellular pH levels in animal tissues, plant cells, bacteria, and yeast. Additionally, BCECF, AM is extensively applied in processes involving intracellular pH changes, such as cytotoxicity, apoptosis, cell adhesion, drug resistance, and chemotaxis.

Experimental Procedure (using human neutrophils as an example):

1. Reagents: 5 mM BCECF, AM/DMSO; HEPES buffer (20 mM HEPES, 153 mM NaCl, 5 mM KCl, 5 mM glucose, pH 7.4).

2. Procedure: BCECF, AM ester in solution can be diluted to the working concentration as needed. The commonly used working concentration is 1-10 μM.

2.1 Prepare a cell suspension (~10^6 cells/mL) using HEPES buffer.

2.2 Add the 5 mM BCECF, AM/DMSO solution to the cell suspension (at a volume of 1/1000 of the cell suspension) to achieve a final concentration of 5 μM BCECF, AM.

2.3 Incubate at 37°C for 30 minutes.

2.4 Wash the cells 2-3 times with HEPES buffer.

2.5 Detect the fluorescence intensity of the cells using a fluorescence microscope or a laser confocal microscope equipped with an image analysis system.

Note: The labeling conditions may vary depending on the cell type. Please determine the optimal conditions before each experiment. The above method is for reference only.

Precautions:

1. BCECF, AM may be harmful to humans. Please take appropriate precautions.
2. BCECF, AM may solidify and adhere to the bottom, walls, or cap of the centrifuge tube at low temperatures (e.g., 4°C or on ice). It can be warmed in a water bath at 20-25°C until fully dissolved before use.
3. Fluorescent dyes are prone to quenching. Please avoid light exposure as much as possible to slow down fluorescence quenching.

4. For your safety and health, please wear a lab coat and disposable gloves during the operation.