BCECF, AM

Catalog number: 21202
Unit size: 1 mg

Component	Storage	Amount
BCECF, AM	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)

OVERVIEW

Intracellular pH plays an important modulating role in many cellular events, including cell growth, calcium regulation, enzymatic activity, receptor-mediated signal transduction, ion transport, endocytosis, chemotaxis, cell adhesion and other cellular processes. pH-sensitive fluorescent dyes have been widely applied to monitor changes in intracellular pH in recent years. Imaging techniques that use fluorescent pH indicators also allow researchers to investigate these processes with much greater spatial resolution and sampling density that can be achieved using other technologies such as microelectrode. Among them, 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein (BCECF) is the most popular pH probe since it can be used to monitor cellular pH ratiometrically. BCECF AM is the cell-permeable version of BCECF.

KEY PARAMETERS

Fluorescence microscope

Excitation	FITC filter set
Emission	FITC filter set
Recommended plate	Black wall/clear bottom

Fluorescence microplate reader

Excitation	490, 430
Emission	535
Cutoff	515
Recommended plate	Black wall/clear bottom
Instrument specification(s)	Bottom read mode

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

BCECF AM Stock Solution
Prepare a 2 to 20 mM stock solution of BCECF AM in high-quality, anhydrous DMSO.

PREPARATION OF WORKING SOLUTION

BCECF AM Working Solution
On the day of the experiment, either dissolve BCECF AM in DMSO or thaw an aliquot of the indicator stock solution to room temperature. Prepare a BCECF AM working solution of 5 to 50 µM in a buffer of your choice (e.g., Hanks and Hepes buffer).

Note The nonionic detergent Pluronic® F-127 can be used to increase the aqueous solubility of AM esters. In the staining buffer, the final Pluronic® F-127 concentration should be approximately 0.02%. A variety of Pluronic® F-127 products can be purchased from AAT Bioquest. Avoid long-term storage of AM esters in the presence of Pluronic® F-127.

Note If your cells contain organic anion-transporters, probenecid (1-2 mM) may be added to the dye working solution (final in well concentration will be 0.5-1 mM) to reduce leakage of the de-esterified indicators. A variety of ReadiUse™ probenecid products, including water-soluble, sodium salt, and stabilized solution, can be purchased from AAT Bioquest.

SAMPLE EXPERIMENTAL PROTOCOL

The following is a recommended protocol for loading BCECF AM into live mammalian cells. This protocol only provides a guideline, should be modified according to your specific needs.

1. Prepare viable cells as desired.
2. On the next day, add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of the BCECF AM working solution into the cell plate.

   Note If your compound(s) interfere with the serum, replace the growth medium with fresh HHBS buffer (100 µL/well for 96-well plate or 25 µL/well for 384-well plate) before dye-loading.
3. Incubate the dye-loaded plate in a cell incubator at 37 °C for 30 to 60 minutes.
4. Replace the dye working solution with HHBS or buffer of your choice to remove any excess probes.
5. Prepare the compound plates using HHBS or a buffer of your choice.
6. Run the pH assay as desired and simultaneously measure fluorescence using either a fluorescence microscope equipped with a FITC filter set or a fluorescence plate reader at Ex/Em = 490/535 nm cutoff 515 nm. For ratio measurements, monitor fluorescence at Ex/Em $_1$ = 430/535 nm cutoff 515 nm and Ex/Em $_2$ = 505/535 nm cutoff 515 nm.

   Note The compound addition is 50 µL/well (96-well plate) or 25 µL/well (384-well plate).

   Note Assays should be completed within 3 to 5 minutes after compound addition. However, a minimum of 8 minutes is recommended for data collection.

EXAMPLE DATA ANALYSIS AND FIGURES

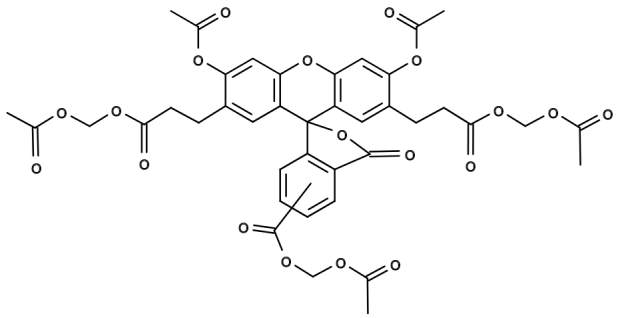

Figure 1. Chemical structure for BCECF, AM

DISCLAIMER

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