BCECF, AM

Ordering Information	*Storage Conditions*
Product Number: 21202 (1 mg)	Keep at -20 °C and avoid light

Introduction

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. AAT Bioquest has developed a better version, and single isomer compound BCFL, AM that make the pH measurement much more reproducible than the BCECF, AM, which is consisted of quite a few different isomers.

Chemical and Physical Properties

Molecular Weight: ~ 808.69
Solvent: dimethylsulfoxide (DMSO)
Spectral Properties: Ex/Em = 503/528 nm

Assay Protocol for Standard Cell Load

Brief Summary

Prepare cells in growth medium→ Add equal volume of BCECF, AM dye-loading solution (100 µL/well for 96-well plate)→ Incubate at 37°C for 1 hour→ Wash and replace with HHBS → Read Fluorescence at Ex/Em= 490/535 nm with 50 uL/well compound addition (or 505/535 and 430/535 nm for ratio)

Note: The following is the recommended protocol for standard cell load. The protocol only provides a guideline, should be modified according to the specific needs.

1. Prepare cells as desired. For example, plate adherent cells overnight in growth medium at 40,000 to 80,000 cells/well/100µL for 96-well or 10,000 to 20,000 cells/well/25µL for 384-well plates.
Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density.

2. Prepare BCECF, AM dye-loading solution:

2.1 Prepare a 2 to 20 mM stock solution of BCECF, AM in high-quality, anhydrous DMSO. The stock solution should be used promptly. Any unused solution need to be aliquoted and refrozen at ≤ -20 °C.
Note: Avoid repeated freeze-thaw cycles, and protect from light.

2.2 Prepare a 5-50 uM BCECF, AM dye-loading solution in Hanks and 20 mM Hepes buffer (HHBS).

3. Run pH Assay

3.1 Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) BCECF, AM dye-loading solution into the cell plate (from Step 2.3).
Note: It is important to replace the growth medium with HHBS buffer (100 µL/well for 96-well plate or 25 µL/well for 384-well plate before dye-loading) if your compounds interfere with the serum.

3.2 Incubate the dye-loading plate at cell incubator for 30 to 60 minutes.

3.3 Wash and replace the dye-loading solution with HHBS.

3.4 Prepare the compound plates by using HHBS or your desired buffer.

3.5 Run the pH assay by monitoring the fluorescence at Ex/Em = 490/535 nm (cut off at 515 nm) or 505/535 nm and 430/535 nm (cut off at 515 nm) for ratio measurements. The compound addition is 50 µL/well (96-well plate) or 25 µL/well (384-well plate).
Note: The assay should be complete within 3 to 5 min after compound addition, however a minimum of 8 min data collection are recommended for during assay development.