RatioWorks™ BCFL, AM *Superior replacement for BCECF*

Catalog number: 21190
Unit size: 1 mg

Component	Storage	Amount
RatioWorks™ BCFL, AM *Superior replacement for BCECF*	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. However, all the commercial BCECF AM is a complex mixture of at least three isomers with different ratios from batch to batch, complicating the BCECF applications. BCFL is developed to overcome this isomer difficulty associated with BCECF AM. As BCECF, BCFL exhibits a pH-dependent dual excitation, essentially identical to BCECF. It has pKa of ~7.0, identical to BCECF too. As with BCECF, the dual excitation spectrum of BCFL with an isosbestic point at 454 nm should make BCFL a good excitation-ratiometrie pH indicator. BCFL ratiometric imaging makes intracellular pH determination essentially independent of several variable factors, including dye concentration, path length, cellular leakage and photobleaching rate. BCFL, AM is a single isomer, making the pH measurement much more reproducible than the BCECF, AM, which is consisted of quite a few different isomers.

KEY PARAMETERS

Fluorescence microscope

Excitation	Single: FITC, Ratio: AmCyan/FITC
Emission	Single: FITC, Ratio: AmCyan/FITC
Recommended plate	Black wall/clear bottom

Fluorescence microplate reader

Excitation	430, 505
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.

RatioWorks™ BCFL AM Stock Solution
Prepare a 10 to 20 mM stock solution of RatioWorks™ BCFL AM in high-quality, anhydrous DMSO.

PREPARATION OF WORKING SOLUTION

RatioWorks™ BCFL AM Working Solution
On the day of the experiment, either dissolve RatioWorks™ BCFL AM in DMSO or thaw an aliquot of the indicator stock solution to room temperature. Prepare a RatioWorks™ BCFL 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 RatioWorks™ BCFL 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 RatioWorks™ BCFL 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

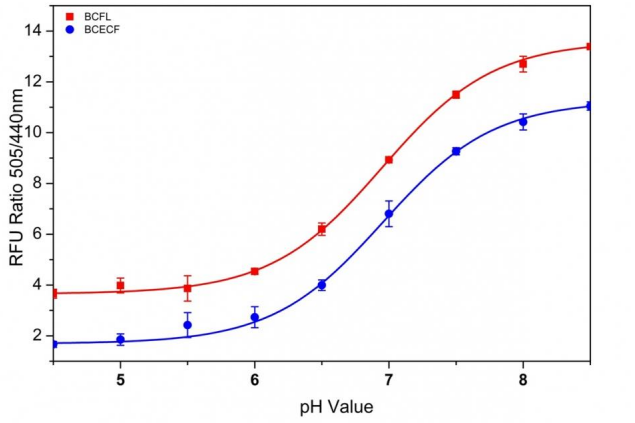

Figure 1. Standard curve created using BCFL, AM (Cat# 21190) and BCECF,AM (Cat#21202) with Intracellular pH Calibration Buffer Kit. Hela cells were incubated with 5 uM BCFL, AM for 30 minutes 37oC. The Intracellular pH Calibration Buffer Kit (Cat#21135) was used to clamp the intracellular pH with extracellular buffers at pH 4.5 to 8.0. Averages of 4 data points were plotted and a 4-parameter trendline was fitted to get the pH standard curve from 6 to 8.

DISCLAIMER

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