RatioWorks™ BCFL Acid *Superior Replacement to BCECF*

Ordering Information	*Storage Conditions*
Product Numbers: 21189 (1 mg)	Keep at -20 °C and desiccated, Avoid exposure to light

Chemical and Physical Properties

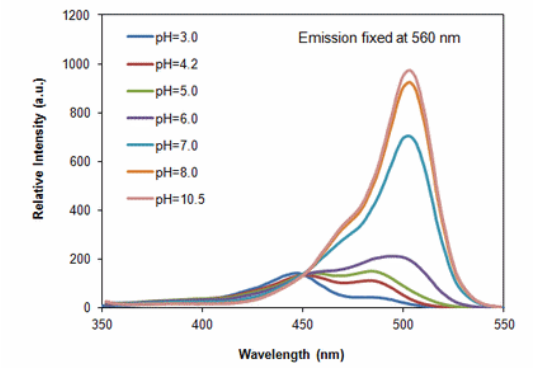
Molecular Weight: ~500
Appearance: Light yellow
Solvents: Water or dimethylsulfoxide (DMSO)
Spectral Properties: Excitation = 503 nm; Emission = 526 nm.

Biological Applications

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. This BCFL is the esterase-hydrolyzed product of BCFL AM that can be used a reference standard with BCFL AM.

Sample Protocol

1). Make 1-10 mM DMSO stock solution. The DMSO stock solution is good for 6 months if stored at -20 °C.
2). Dilute it into different pH buffer at concentration of 10 to 50 uM.
3). Measure the excitation spectra with a fixed emission at 560 nm.



References

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3. Bachmeier CJ, Trickler WJ, Miller DW. (2004) Drug efflux transport properties of 2',7'- bis(2-carboxyethyl)-5(6)-carboxyfluorescein acetoxymethyl ester (BCECF-AM) and its fluorescent free acid, BCECF. J Pharm Sci, 93, 932.
4. Ozkan P, Mutharasan R. (2002) A rapid method for measuring intracellular pH using BCECF-AM. Biochim Biophys Acta, 1572, 143.

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