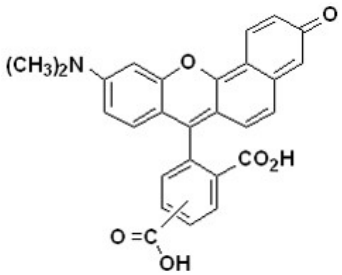
FT-36969A

RhodFluor pH Indicator

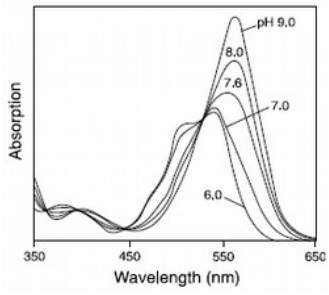
Visible light–excitable fluorescent pH indicators based on seminaphthorhodafluors

Product Description

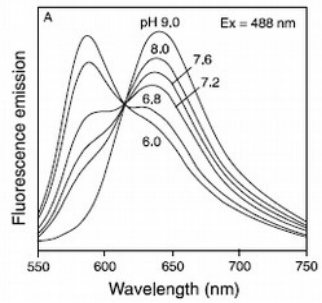
Product cat.number *	Structure	MW	Solubility	Storage Application
5-(6)-Carboxy RhodFluor FP-36969A, 1mg [126208-12-6]	$C_{27}H_{19}NO_6$	453,45	DMSO, DMF and 5% Methanol/chloroform	Room temperature
5-(6)-Carboxy RhodFluor, Acetate, Succinimidyl Ester FP-99986A, 20x50µg FP-99986B, 1mg	$C_{33}H_{24}N_2O_9$	592,56	DMSO, DMF, Acetonitrile and Chloroform	Store at -20°C Long-term cell tracing with orange-red fluorescence that can be easily distinguished from that of cells loaded with green-fluorescent tracers such as CFDA SE.
5-(6)-Carboxy RhodFluor, acetoxymethyl ester, acetate FP-834733, 20x50µg FP-834734, 1mg [126208-13-7]	$C_{32}H_{25}NO_9$	567,55	DMSO, DMF, Acetonitrile and Chloroform	Store at -20°C
5-(6)-Carboxy RhodFluor, Succinimidyl Ester FP-1A5870, 1mg	$C_{31}H_{22}N_2O_8$	567,55	DMSO, DMF and 5% Methanol/chloroform	Store at -20°C Long-term cell tracing



5-(6)-Carboxy RhodFluor



Absorption spectra



Emission spectra

Introduction

5-(6)-Carboxy RhodFluor exhibits a significant pH-dependent emission shift from yellow-orange to deep red fluorescence under acidic and basic conditions, respectively. This pH dependence allows the ratio of the fluorescence intensities from the dye at two emission wavelengths – typically 580 nm and 640 nm – to be used for quantitative determinations of pH. The exciting is at one wavelength, between 488 nm and 530 nm. The pKa of ~7.5 after de-esterification is useful for measuring pH changes between pH 7 and pH 8.

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Simultaneous measurements of intracellular pH and calcium have been made using 5-(6)-Carboxy RhodFluor together with fura-2, fluo-3 and indo-1. The long-wavelength emission from 5-(6)-Carboxy RhodFluor is also useful for studies that employ DIDS (# FP-46770A), amilorides or other modifiers of cell function that can introduce background fluorescence at shorter wavelengths. Amine-reactive RhodFluor succinimidyl esters are designed for longterm cellular retention via coupling to proteins.

Directions for use

Storage

Upon receipt, 5-(6)-Carboxy RhodFluor activiated forms should be stored frozen at ≤.20°C, desiccated and protected from light. Stock solutions of 5-(6)-Carboxy RhodFluor are typically prepared at 1-10 mM in high-quality anhydrous dimethyl sulfoxide (DMSO). Al though we recommend that this stock solution be prepared immediately before use, it may be stored by dividing it into single-use aliquots and freezing the aliquots at ≤.20°C, protected from light. Aqueous solutions should be discarded at the end of the day.

Guidelines for use

Optimal loading conditions for each cell type and experiment should be determined by the researcher. The literature cites a wide range of loading conditions, from 1 to 20 µM 5-(6)-Carboxy RhodFluor incubated with cells for from 10 to 60 minutes. DMSO stock so lu tions are typically diluted at least 1:1000 into loading buffer to reduce the exposure of cells to DMSO, and the loading buffer should be serum-free because serum often contains esterase activity. The non ionic detergent Pluronic® F-127 is sometimes used to promote dispersion of the rather non polar 5-(6)-Carboxy RhodFluor acetate esters into buffers.

As initial loading conditions, we recommend in cu bat ing cells in 1$\frac{3}{8}$10 µM 5-(6)-Carboxy RhodFluor for 30 minutes at the opti mum temperature for the specific cell type of interest. After loading, cells should be washed before commencing pH measurements. For loading brain slices, incubation for 60 minutes in artificial cerebrospinal fluid (ACSF) contain ing 20 µM 5-(6)-Carboxy RhodFluor AM acetate and 4% (w/v) Pluronic F-127 followed by a further 30 minute incubation in dye-free ACSF is recommended.

Nigericin is an antibiotic derived from *Streptomyces hygroscopicus.* None-Fluorescent potassium ionophore, nigericin is usually used to equilibrate the pH inside and outside the cell. Nigericin acts as an H+, K+, Pb2+ ionophore. For calibration of 5-(6)-Carboxy RhodFluor, a concentration of 10-50 µM of Nigericin in the presence of 100-150mM K+ to equilibrate the intracellular pH with the controlled extracellular medium. The pH-dependent spec tral shifts exhibited by 5-(6)-Carboxy RhodFluor al low calibration of the pH response in terms of the ratio of fluorescence intensities measured at two different wavelengths (equation 1). R is the ratio $F_{\lambda 1}/F_{\lambda 2}$ of fluorescence intensities (F) measured at two wave lengths λl and λ2 and the sub scripts A and B represent the limiting values at the acidic and basic end points of the titration respectively.

$$[H+] = K_a\left(\frac{R - R_B}{R_A - R}\right) \times \frac{F_{B(\lambda 2)}}{F_{A(\lambda 2)}} \qquad (1)$$

A number of fluorescence measurement artifacts are eliminated with this ratiometric method, including photobleaching, cell thickness, instrument stability and leakage and non uniform loading of the indicator. Note that background fluores cence corrections should be subtracted before calculation of R. 5-(6)-Carboxy RhodFluor offers a large number of options for selection of λ1 and λ2. A typical calibration would use a dual-emission ratio with λ1 = 580 nm and λ2 = 640 nm and fixed excitation at 514 nm. Note that selection of λ2 at the pH-independent isosbestic point (~600 nm for carboxy RhodFluor) eliminates the normalization factor $F_{B(\lambda 2)}/F_{A(\lambda 2)}$ from equation (1).

The logarithmic form of equation (1) is:

$$pH = pK_A - \log\left[\frac{R - R_B}{R_A - R} \times \frac{F_{B(\lambda 2)}}{F_{A(\lambda 2)}}\right] \qquad (2)$$

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In this form, the data should yield a linear plot with a slope of 1 and an intercept equal to the pKa.

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Technical and scientific information

Related products

- Pluronic® F-127, FP-37361A
- Pluronic® F-127, 10% solution in sterile water , FP-379951
- Nigericin, FP-474529

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