**RhodFluor pH Indicator**

Visible light–excitable fluorescent pH indicators based on seminaphthorhodafluors

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### Product Description

<table>
<thead>
<tr>
<th>Product name</th>
<th>cat. number</th>
<th>Structure</th>
<th>MW</th>
<th>Solubility</th>
<th>Storage Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-(6)-Carboxy RhodFluor</td>
<td>FP-36969A, 1mg [126208-12-6]</td>
<td>C_{27}H_{19}NO_{6}</td>
<td>453,45</td>
<td>DMSO, DMF and 5% Methanol/chloroform</td>
<td>Room temperature</td>
</tr>
<tr>
<td>5-(6)-Carboxy RhodFluor, Acetate, Succinimidyl Ester</td>
<td>FP-99986A, 20x50µg, FP-99986B, 1mg</td>
<td>C_{33}H_{24}N_{2}O_{9}</td>
<td>592,56</td>
<td>DMSO, DMF, Acetonitrile and Chloroform</td>
<td>Store at -20°C</td>
</tr>
<tr>
<td>5-(6)-Carboxy RhodFluor, acetoxymethyl ester, acetate</td>
<td>FP-834733, 20x50µg, FP-834734, 1mg [126208-13-7]</td>
<td>C_{32}H_{25}NO_{9}</td>
<td>567,55</td>
<td>DMSO, DMF, Acetonitrile and Chloroform</td>
<td>Store at -20°C</td>
</tr>
<tr>
<td>5-(6)-Carboxy RhodFluor, Succinimidyl Ester</td>
<td>FP-1A5870, 1mg</td>
<td>C_{31}H_{22}N_{2}O_{8}</td>
<td>567,55</td>
<td>DMSO, DMF and 5% Methanol/chloroform</td>
<td>Store at -20°C</td>
</tr>
</tbody>
</table>

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### 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.
Simultaneous measurements of intracellular pH and calcium have been made using 5-(6)-Carboxy RhodFlor together with fura-2, fluo-3 and indo-1. The long-wavelength emission from 5-(6)-Carboxy RhodFlor 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 RhodFlor succinimidyl esters are designed for longterm cellular retention via coupling to proteins.

**Directions for use**

**Storage**

Upon receipt, 5-(6)-Carboxy RhodFlor activated forms should be stored frozen at ≤.20°C, desiccated and protected from light. Stock solutions of 5-(6)-Carboxy RhodFlor are typically prepared at 1-10 mM in high-quality anhydrous dimethyl sulfoxide (DMSO). Although 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 RhodFlor incubated with cells for from 10 to 60 minutes. DMSO stock solutions 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 RhodFlor acetate esters into buffers.

As initial loading conditions, we recommend incubating cells in 10-100 µM 5-(6)-Carboxy RhodFlor for 30 minutes at the optimum 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) containing 20 µM 5-(6)-Carboxy RhodFlor 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 RhodFlor, 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 spectral shifts exhibited by 5-(6)-Carboxy RhodFlor at 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 $F_{λ1}/F_{λ2}$ of fluorescence intensities (F) measured at two wave lengths $λ1$ and $λ2$ and the subscripts A and B represent the limiting values at the acidic and basic end points of the titration respectively.

$$[H+] = K_A \left( \frac{R - R_B}{R_A - R} \right) \times \frac{F_{λ2}}{F_{λ1}} \quad (1)$$

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 fluorescence corrections should be subtracted before calculation of R. 5-(6)-Carboxy RhodFlor 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 RhodFlor ) eliminates the normalization factor $F_{λ2}/F_{λ1}$ from equation (1).

The logarithmic form of equation (1) is:

$$pH = \log \left( \frac{R - R_B}{R_A - R} \right) \times \frac{F_{λ2}}{F_{λ1}} \quad (2)$$
In this form, the data should yield a linear plot with a slope of 1 and an intercept equal to the pKa.

References

- Exposure of cells to hydrogen peroxide can increase the intracellular accumulation of drugs. Funk RS, Krise JP, Mol Pharm (2007) 4:154-159

Technical and scientific information

Related products

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

Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com. Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06

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