# SNARF® pH Indicators

## Introduction

Carboxy SNARF®-1 is a long-wavelength fluorescent pH indicator developed by Molecular Probes.[1,2] The emission spectrum of carboxy SNARF-1 undergoes a pH-dependent wavelength shift, thus allowing the ratio of the fluorescence intensities from the dye at two emission wavelengths to be used for more accurate determinations of pH. Carboxy SNARF-1 is typically used by exciting the dye at one wavelength, between 488 nm and 530 nm, while monitoring the fluorescence emission at two wavelengths, typically about 580 nm and 640 nm (Figures 1 and 2).

Carboxy SNARF-1 is particularly well suited for instrumentation with a visible-light fixed-wavelength excitation source, including confocal laser scanning microscopes,[3] flow cytometers [4] and fluorescence microplate readers.[5] The dye is efficiently excited by both the 488 nm and 514 nm lines of the argon-ion laser. Simultaneous measurements of intracellular pH and calcium have been made using carboxy SNARF-1 together with fura-2,[6] fluo-3 [7] and indo-1.[8] The long-wavelength emission from carboxy SNARF-1 is also useful for studies that employ DIDS, amilorides or other modifiers of cell function that can introduce background fluorescence at shorter wavelengths.[9] SNARF-4F and SNARF-5F are fluorinated derivatives of carboxy SNARF-1 with lower pH sensitivity maxima. For improved cellular retention, we offer high molecular weight dextran conjugates of SNARF-1 (D-3303, D-3304), which must be loaded into cells by microinjection, electroporation or comparable techniques.[10] Amine-reactive SNARF-1 carboxylic acid, acetate, succinimidyl ester and thiol-reactive 5-(and-6)-chloromethyl SNARF-1 acetate are designed for long-term cellular retention via coupling to proteins.

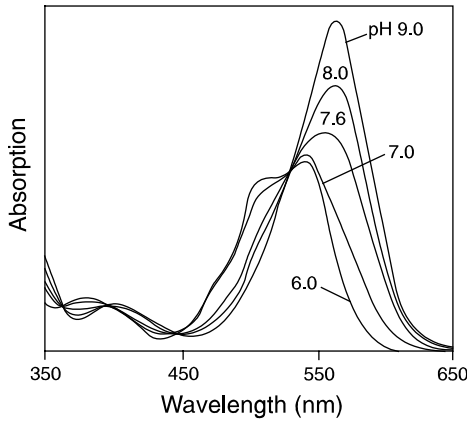


**Figure 1.** Absorption spectra of carboxy SNARF-1 dissolved in 50 mM potassium phosphate buffers at various pH values.

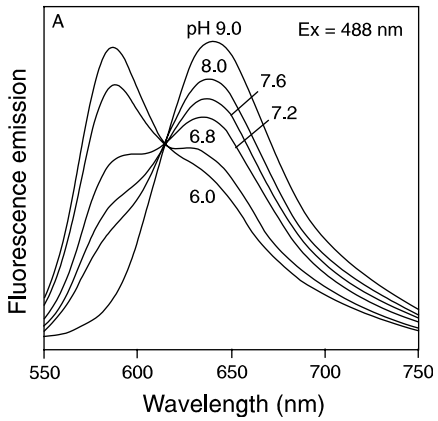


**Figure 2.** Emission spectra of carboxy SNARF-1 in 50 mM potassium phosphate buffers at various pH values. Samples were excited at 488 nm.

## Contents and Storage

Carboxy SNARF-1 (C-1270) is provided as a lyophilized solid in units of 1 mg. Upon receipt, it can be stored protected from light at room temperature, 2–6°C or ≤–20°C without compromising stability. Stock solutions can be made in water or buffer and should be divided into aliquots and stored frozen at ≤–20°C, protected from light.

Carboxy SNARF-1 AM acetate is provided as a lyophilized solid in units of 1 mg (C-1271) or specially packaged in 20 vials, each containing 50 µg (C-1272). Upon receipt, carboxy SNARF-1 AM acetate should be stored frozen at ≤–20°C, desiccated and protected from light. Because carboxy SNARF-1 AM acetate is susceptible to hydrolysis, it must be protected from moisture during storage. Stock solutions of carboxy SNARF-1 AM acetate (molecular weight = 568) are typically prepared at 1–10 mM in high-quality anhydrous dimethylsulfoxide (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 of carboxy SNARF-1 AM acetate should be discarded at the end of the day.

SNARF-1 carboxylic acid, acetate, succinimidyl ester (S-22801) is supplied in sets of 10 × 50 µg vials of lyophilized solid. 5-(and-6)-chloromethyl SNARF-1 acetate (C-6826) is supplied in sets of 20 × 50 µg vials. Like carboxy SNARF-1 AM acetate, these products are susceptible to hydrolysis and must be protected from moisture during storage.

Dextran conjugates should be stored desiccated and protected from light at ≤–20°C. These conjugates can be dissolved in user-specified intracellular injection buffers at 10 mM (=100 mg/mL for a 10,000 MW dextran) for microinjection into cells.

## Application

### Loading Cells with SNARF AM Acetate Esters

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 SNARF AM acetate 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 nonionic detergent Pluronic® F-127 is sometimes used to promote dispersion of the rather nonpolar SNARF AM acetate esters into buffers. For the convenience of our customers, Molecular Probes offers Pluronic F-127 in three forms: 1 mL of a 20% (w/v) solution in DMSO (P-3000), 30 mL of a sterile 10% (w/v) solution in water (P-6866) and 2 g solid (P-6867). As initial loading conditions, we recommend incubating cells in 1–10 µM SNARF AM acetate 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 carboxy SNARF-1 AM acetate and 4% (w/v) Pluronic F-127 followed by a further 30 minute incubation in dye-free ACSF is recommended.[11]

### Calibration

Calibrating the fluorescence response of SNARF indicators *in vitro* with pH-controlled buffers yields $pK_a$ values of ~7.5 for carboxy SNARF-1, ~6.4 for SNARF-4F and ~7.2 for SNARF-5F. However, because the response is usually significantly different when the dye is loaded in cells,[12,13] *in situ* calibration is generally advisable for each experimental system. *In situ* calibration can be performed by using the ionophore nigericin (N-1495) at a concentration of 10–50 µM in the presence of 100–150 mM $K^+$ to equilibrate the intracellular pH with the controlled extracellular medium.[12,14] The pH-dependent spectral shifts exhibited by carboxy SNARF-1 (Figures 1 and 2) allow 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 wavelengths λl and λ2 and the subscripts A and B represent the limiting values at the acidic and basic endpoints of the titration respectively.

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

A number of fluorescence measurement artifacts are eliminated with this ratiometric method, including photobleaching, cell thickness, instrument stability and leakage and nonuniform loading of the indicator. Note that background fluorescence corrections should be subtracted *before* calculation of R. Carboxy SNARF-1 offers a large number of options for selection of $\lambda_1$ and $\lambda_2$. A typical calibration would use a dual-emission ratio with $\lambda_1$ = 580 nm and $\lambda_2$ = 640 nm and fixed excitation at 514 nm. Note that selection of $\lambda_2$ at the pH-independent isosbestic point (~600 nm for carboxy SNARF-1) eliminates the normalization factor $F_{B(\lambda 2)}/F_{A(\lambda 2)}$ from equation (1).

The logarithmic form of equation (1) is:

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

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

## References


**1.** Anal Biochem 194, 330 (1991); **2.** US Patent 4,945,171; **3.** Am J Physiol 275, H1937 (1998); **4.** Cytometry 12 127 (1991); **5.** Am J Physiol 273, C1783 (1997); **6.** Am J Physiol 260, C297 (1991); **7.** Cytometry 14, 916 (1993); **8.** Am J Physiol 261, H1671 (1991); **9.** Pflügers Arch 417, 234 (1990); **10.** Science 263, 1419 (1994); **11.** Methods 18, 150 (1999); **12.** Anal Biochem 204, 65 (1992); **13.** J Fluorescence 2, 75 (1992); **14.** Methods Enzymol 192, 38 (1990).


## Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

| Cat # | Product Name | Unit Size |
|---|---|---|
| C-1270 | 5-(and-6)-carboxy SNARF®-1 | 1 mg |
| C-1271 | 5-(and-6)-carboxy SNARF®-1, acetoxymethyl ester, acetate | 1 mg |
| C-1272 | 5-(and-6)-carboxy SNARF®-1, acetoxymethyl ester, acetate *special packaging* | 20 x 50 µg |
| C-6826 | 5-(and-6)-chloromethyl SNARF®-1, acetate *mixed isomers* *special packaging* | 20 x 50 µg |
| D-3303 | dextran, SNARF®-1, 10,000 MW, anionic | 5 mg |
| D-3304 | dextran, SNARF®-1, 70,000 MW, anionic | 5 mg |
| N-1495 | nigericin, free acid | 10 mg |
| P-6867 | Pluronic® F-127 *low UV absorbance* | 2 g |
| P-3000 | Pluronic® F-127 *20% solution in DMSO* | 1 mL |
| P-6866 | Pluronic® F-127 *sterile 10% solution in water* | 30 mL |
| S-22801 | SNARF®-1 carboxylic acid, acetate, succinimidyl ester *special packaging* | 10 x 50 µg |
| S-23920 | SNARF®-4F 5-(and-6)-carboxylic acid *cell impermeant* | 1 mg |
| S-23921 | SNARF®-4F 5-(and-6)-carboxylic acid, acetoxymethyl ester, acetate *cell permeant* *special packaging* | 20 x 50 µg |
| S-23922 | SNARF®-5F 5-(and-6)-carboxylic acid *cell impermeant* | 1 mg |
| S-23923 | SNARF®-5F 5-(and-6)-carboxylic acid, acetoxymethyl ester, acetate *cell permeant* *special packaging* | 20 x 50 µg |

## *Contact Information*

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

Please visit our Web site — **www.probes.com** — for the most up-to-date information.


**Molecular Probes, Inc.**
29851 Willow Creek Road, Eugene, OR 97402
Phone: (541) 465-8300 • Fax: (541) 344-6504

**Customer Service:** 6:00 am to 4:30 pm (Pacific Time)
Phone: (541) 465-8338 • Fax: (541) 344-6504 • order@probes.com

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**Molecular Probes Europe BV**
PoortGebouw, Rijnsburgerweg 10
2333 AA Leiden, The Netherlands
Phone: +31-71-5233378 • Fax: +31-71-5233419

**Customer Service:** 9:00 to 16:30 (Central European Time)
Phone: +31-71-5236850 • Fax: +31-71-5233419
eurorder@probes.nl

**Technical Assistance:** 9:00 to 16:30 (Central European Time)
Phone: +31-71-5233431 • Fax: +31-71-5241883
eurotech@probes.nl