

## Nitric Oxide Indicators: DAF-FM and DAF-FM Diacetate

**D-23841 DAF-FM (4-amino-5-methylamino- 2',7'-difluorescein)**

**D-23842 DAF-FM diacetate (4-amino-5-methylamino- 2',7'-difluorofluorescein diacetate)**

**D-23844 DAF-FM diacetate (4-amino-5-methylamino- 2',7'-difluorofluorescein diacetate) \*special packaging\***

### Quick Facts

#### Storage upon receipt:

- -20°C
- Desiccate
- Protect from light

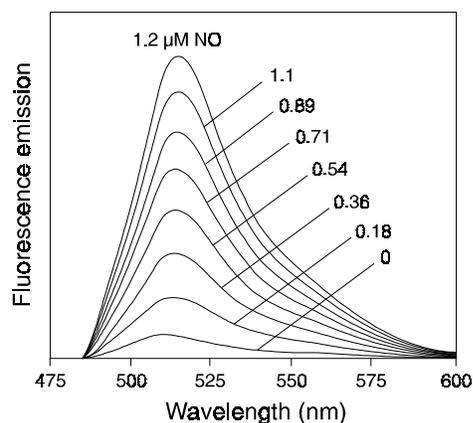
**Ex/Em:** 495/515

Probably the most successful indicator for nitric oxide has been 4,5-diaminofluorescein diacetate (DAF-2 diacetate),<sup>1,2</sup> which was also developed by Kojima and collaborators. DAF-2 has been used to identify individual nitric oxide-producing neurons in brain slices,<sup>4,5</sup> in mitochondria<sup>6</sup> and in living plant cells.<sup>7</sup> Simultaneous measurements of intracellular  $\text{Ca}^{2+}$  with fura-2 and nitric oxide production with DAF-2 have been reported.<sup>8</sup>

The DAF-FM reagent has some important advantages over DAF-2. The spectra of the NO adduct of DAF-FM are independent of pH above pH 5.5.<sup>3</sup> Also, the NO adduct of DAF-FM is significantly more photostable than that of DAF-2,<sup>3</sup> which means additional time for image capture. Finally, DAF-FM is a more sensitive reagent for NO than is DAF-2 (NO detection limit for DAF-FM  $\sim 3 \text{ nM}$ <sup>3</sup> versus  $\sim 5 \text{ nM}$  for DAF-2<sup>2</sup>).

### Introduction

DAF-FM (D-23841) and DAF-FM diacetate (D-23842) represent two important new reagents for quantitating low concentrations of nitric oxide (Figure 1). Developed by Kojima and collaborators,<sup>1,2</sup> these compounds are essentially nonfluorescent until they react with NO to form a fluorescent benzotriazole (Figure 2). DAF-FM diacetate is cell-permeant and passively diffuses across cellular membranes. Once inside cells, it is deacetylated by intracellular esterases to become DAF-FM. The fluorescence quantum yield of DAF-FM is  $\sim 0.005$ , but increases about 160-fold, to  $\sim 0.81$ , after reacting with nitric oxide.<sup>3</sup> With excitation/emission maxima of 495/515 nm, DAF-FM can be detected by any instrument that can detect fluorescein, including flow cytometers, microscopes, fluorescent microplate readers and fluorimeters.



**Figure 1.** Fluorescence emission spectra of DAF-FM in solutions containing zero to 1.2  $\mu\text{M}$  nitric oxide (NO) radical.

### Storage and Handling

Upon receipt DAF-FM (D-23841) and DAF-FM diacetate (D-23841, D-23844) should be stored at  $-20^\circ\text{C}$ , desiccated and protected from light.

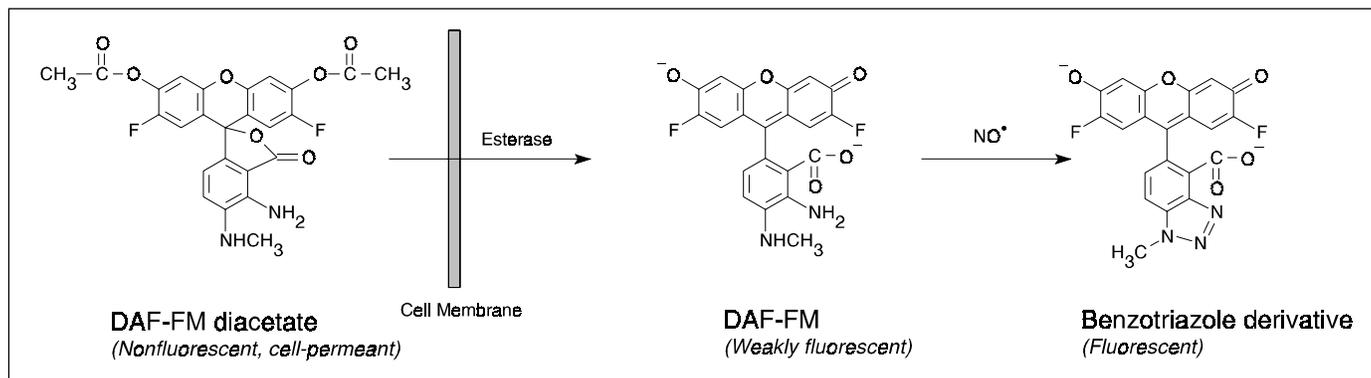
A  $\sim 7 \text{ mM}$  stock solution of DAF-FM (MW = 412) can be prepared by dissolving the entire contents of the vial in 0.35 mL of high-quality anhydrous DMSO.

A  $\sim 5 \text{ mM}$  stock solution of DAF-FM diacetate (MW = 496) can be made by dissolving the 1 mg packaging (D-23841) in 0.4 mL of high-quality anhydrous DMSO or the 50  $\mu\text{g}$  packaging (D-23844) in 20  $\mu\text{L}$  of high-quality anhydrous DMSO.

For long-term storage of the DMSO stock solutions, divide the solution into aliquots in order to minimize freeze-thaw cycles. These aliquoted solutions should be stable for at least six months. Please allow the solutions to warm to room temperature before opening. Working solutions of these reagents should be prepared immediately before use. The diluted reagent should not be stored for later use.

### Application

Both the DAF-FM and the diacetate DMSO stock solutions can be diluted into aqueous buffers. Bovine serum albumin (BSA) and phenol red may affect the fluorescence and should be used with caution. DAF-FM and DAF-FM diacetate are sold for research use only. They should not be used for *in vitro* diagnostics.



**Figure 2.** Reaction scheme for the detection of nitric oxide (NO) by DAF-FM and DAF-FM diacetate.

### DAF-FM

The optimal dilution buffer and working concentration should be determined empirically. A suggested starting concentration range is between 1–10  $\mu\text{M}$ .

DAF-FM can be loaded into cells by pressure injection or perfusion from a patch-clamp pipette. With these methods it is advisable to use a dead-cell stain, such as propidium iodide, to identify cells that do not recover from the loading procedure. Fluorescence excitation and emission maxima are 495 and 515 nm, respectively. Because these wavelengths are very similar to fluorescein, detection systems designed for fluorescein or FITC can be used.

### DAF-FM Diacetate

The following loading protocol is provided as an introductory guide. Optimal loading concentration, time and temperature will need to be determined empirically. In general, it is desirable to use the minimum dye concentration required to yield fluorescence signals with adequate signal to noise ratios. Subcellular compartmentalization is usually lessened by lowering the incubation temperature.

**1.1** Prepare viable cells in suspension or on a slide.

**1.2** Dilute the DMSO stock solution into a suitable buffer. A suggested starting concentration range is between 1–10  $\mu\text{M}$ .

**1.3** Incubate the cells with the diluted DAF-FM diacetate for 20–60 minutes at 4°C to 37°C. Adherent cultures do not need to be trypsinized for loading.

**1.4** Wash the cells to remove excess probe. Replace with fresh buffer or medium, and then incubate for an additional 15–30 minutes to allow complete de-esterification of the intracellular diacetates.

**1.5** Fluorescence excitation and emission maxima are 495 and 515 nm, respectively. Because these wavelengths are very similar to fluorescein, detection systems designed for fluorescein or FITC can be used.

## References

1. Chem Pharm Bull 46, 373 (1998);
2. Anal Chem 70, 2446 (1998);
3. Angew Chem Int Ed Engl 21, 3209 (1999);
4. J Neurosci Methods 92, 101 (1999);
5. Brain Res 852, 239 (2000);
6. Biochem Biophys Res Commun 272, 129 (2000);
7. Plant J 23, 817 (2000);
8. Cell Calcium 27, 281 (2000).

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

Cat #	Product Name	Unit Size
D-23841	DAF-FM (4-amino-5-methylamino-2',7'-difluorofluorescein) .....	1 mg
D-23842	DAF-FM diacetate (4-amino-5-methylamino-2',7'-difluorofluorescein diacetate) .....	1 mg
D-23844	DAF-FM diacetate (4-amino-5-methylamino-2',7'-difluorofluorescein diacetate) *special packaging* .....	10 x 50 $\mu\text{g}$

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