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

Product	Cat#	Package size
DAF-FM (4-amino-5-methylamino-2',7'-difluorescein	S5449.0001	1mg
DAF-FM (4-amino-5-methylamino-2',7'-difluorescein	S5449.0005	5mg
DAF-FM DA (4-amino-5-methylamino-2',7'-difluorescein diacetate	S5450.0001	1mg
DAF-FM DA (4-amino-5-methylamino-2',7'-difluorescein diacetate	S5450.0005	5mg

Prodcut Description

DAF-FM (S5449) and DAF-FM diacetate (S5450) represent two important new reagents for quantification of low concentrations of nitric oxide. Developed by Kojima and collaborators,[1,2] these compounds are essentially nonfluorescent until they react with NO to form a fluorescent benzotriazole. DAF-FM diacetate is cell-permeable 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 approx. 0.005, but increases about 160-fold, to about 0.81, after reacting with nitric oxide.[3] 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 fluorometers.

Probably the most successful indicator for nitric oxide has been 4,5-diaminofluorescein diacetate (DAF-2 diacetate / S5440),[1,2] which was also developed by Kojima and collaborators. DAF-2 has been used to identify individual nitric oxide-producing neurons in brain slices,[4,5] in mitochondria [6] and in living plant cells.[7] Simultaneous measurements of intracellular Ca2+ with fura-2 and nitric oxide production with DAF-2 have been reported.[8]
DAF-FM has some important advantages over DAF-2. The spectra of the NO adduct of DAF-FM are independent of pH above pH 5.5.[3] Also, the NO adduct of DAF-FM is significantly more photostable than that of DAF-2,[3] 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 ~3nM[3] versus ~5nM for DAF-2[2]).

DAF-FM

The optimal dilution buffer and working concentration should be determined empirically. A uggested starting concentration range is between 1-10 µM.
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 (M3181), 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.

Cell loading protocoll

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 µM.

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.

* 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.

Specifications:

- Ex/Em of DAF-FM: ~495/515 nm.
- Lyophilized product should be dissolved using DMSO and then added to an aqueous buffer to create a working solution.
- DAF-FM diacetate is cell permeant and passively diffuses across cellular membranes; once inside the cell, it is converted to a cell-impermeant form.
- Buffers containing bovine serum albumin (BSA) and phenol red may affect the fluorescence and should be used with caution.
- The fluorescence quantum yield of DAF-FM is ~0.005, but increases about 160-fold, to ~0.81, after reacting with NO.

Applications:

- Assessment of NO production in transaldolase-deficient lymphoblasts by flow cytometry.
- Detection of NO accumulation in embryonic cortical neurons following neurotrophin stimulation.
- in vivo imaging of NO in zebrafish.
- Intravital microscopic detection of NO generation associated with angiogenesis in mice.
- Quantitation of ATP-induced NO release in rabbit platelets

Advantages of DAF-FM over DAF-2

The spectra of the NO adduct of DAF-FM are independent of pH above pH 5.5. Also, the NO adduct of DAF-FM is significantly more photostable than that of DAF-2, 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 ~3nM versus ~5nM for DAF-2).

Shipping, Storage and Handling

The product is shipped at RT. Upon receipt DAF-FM (S5449) and DAF-FM diacetate (S5450) should be stored at -20°C, desiccated and protected from light. A ~7mM stock solution of DAF-FM (MW = 412) can be prepared by dissolving the entire contents of the vial in 0.35mL of high-quality anhydrous DMSO. A ~5mM stock solution of DAF-FM diacetate (MW = 496) can be made by dissolving the 1mg packaging (S5450.0001) in 0.4mL 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.

Certifications

Genaxxon bioscience is ISO 9001:2008 certified. Each stage of the manufacturing process is controlled and monitored by stringent quality control procedures to guarantee the highest possible quality and lot-to-lot reproducibility.

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).

Handling

Good laboratory practice should be employed in the safe handling of any biochemical product. If you are not fully trained or are unaware of the hazards involved, do not use this compound!
Caution: do not take internally! Avoid contact by all modes of exposure. Wear appropriate laboratory attire including a lab coat, gloves, mask and safety glasses. Do not mouth pipette, inhale, ingest or allow coming into contact with open wounds. Wash thoroughly any area of the body which comes into contact with the product. Avoid accidental auto inoculation by exercising extreme care when handling in conjunction with any injection device.

This product is intended for research usage by qualified personnel only. It is not intended for use in humans or animals or as a diagnostic agent. Genaxxon bioscience is not liable for any damages resulting from misuse or handling of this product.