

Product Data Sheet

Product Name: DAF-2
Cat. No.: GC43372

Chemical Properties

Cas. No. 205391-01-1

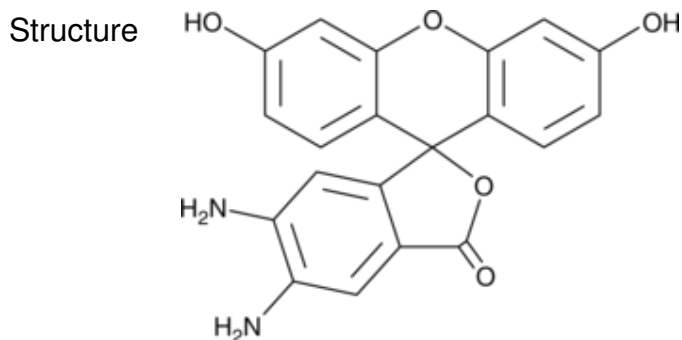
SMILES OC1=CC2=C(C=C1)C3(C(C=C(N)C(N)=C4)=C4C(O3)=O)C5=CC=C(O)C=C5O2

Formula $C_{20}H_{14}N_2O_5$ M.Wt 362.3

Solubility A solution in DMSO Storage Store at -20°C

General tips For obtaining a higher solubility, please warm the tube at 37 °C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution: ship with blue ice All other available size: ship with RT, or blue ice upon request.



Protocol

This plan only provides a guide, please modify it to meet your specific needs.

1. This product is provided in the form of DMSO stock solution with a concentration of 1mM. It is not recommended to change the solvent;
2. It is recommended that unused stock solutions be aliquoted and stored in the dark at -20°C or -80°C to avoid repeated freezing and thawing;
3. Please centrifuge briefly before using the dye to ensure that the product is concentrated at the

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bottom of the tube;

4. BSA and phenol red interfere with the detection of DAF-2. It is recommended to use a suitable buffer (such as serum- and phenol red-free medium or PBS) to dilute the stock solution and prepare a working solution with a concentration of 1-10 μ M;
5. The diluted water-soluble solution should not be stored for more than one day. Please adjust the concentration of the working solution according to the actual situation and make it ready for use;
6. Microscope detection: The maximum excitation/emission wavelength of DAF-2 is 485/538nm.
7. DAF-2 is not cell membrane permeable and can be used for direct detection of nitric oxide in extracellular or solution systems. If you need to detect intracellular nitric oxide, you can choose DAF-2 DA (GC30702), a cell membrane-permeable DAF-2 derivative;
8. Fluorescent dyes all have quenching problems. Please avoid light as much as possible to slow down fluorescence quenching.
9. For your safety and health, please wear a lab coat and disposable gloves.

Background

DAF-2 is a fluorescent detector for nitric oxide (NO) in cells and tissues with excitation and emission wavelengths of 495 and 517nm, respectively. In oxygenated solutions, reactive nitrogen species derived from NO autoxidation nitrosate DAF-2 to yield the highly fluorescent DAF-2 triazole^[1]. The ability of DAF-2 to detect NO was strongly affected by the divalent cation concentration in the medium especially calcium, as well as the incident light^[2]. Once DAF-2 is fully excited, the fluorescence intensity is very stable for more than 30min but the existence of catecholamines and reducer like ascorbate attenuate the NO-induced fluorescence of DAF-2 mainly through an anti-oxidative action^[3].

In vitro, DAF-2 (5 μ M) reliably quantified 4-bromo-A23187-stimulated NO from human EA.hy 926 endothelial cells after incubated 45min at 37°C. The fluorescence increase was detected immediately by HPLC-FLD^[4]. 1mM DAF-2 incubated coronal slices of midbrain or hippocampus isolated from young adult rats for 30min at 33°C. Illumination at 450–490nm revealed punctate fluorescence in neurones in the lateral tegmental nucleus, dorsal raphe nucleus, dorsolateral periaqueductal grey

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matter, deep collicular layers and cortical areas^[5].

References:

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- [2] Broillet M, Randin O, Chatton J. Photoactivation and calcium sensitivity of the fluorescent NO indicator 4,5-diaminofluorescein (DAF-2): implications for cellular NO imaging. *FEBS Lett.* 2001 Mar 2;491(3):227-32.
- [3] Nagata N, Momose K, Ishida Y. Inhibitory effects of catecholamines and anti-oxidants on the fluorescence reaction of 4,5-diaminofluorescein, DAF-2, a novel indicator of nitric oxide. *J Biochem.* 1999 Apr;125(4):658-61.
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- [5] Brown L A, Key B J, Lovick T A. Bio-imaging of nitric oxide-producing neurones in slices of rat brain using 4,5-diaminofluorescein. *J Neurosci Methods.* 1999 Oct 15;92(1-2):101-10.

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