



Cell Technology, Inc



## Nitric Oxide Synthase (NOS) Detection Reagent

### Key Benefits

- Cell permeable
- No wash homogenous assay
- Adaptable to High throughput assay platforms
- Real time detection of NOS activity.

### Assay Principle

Diaminofluorescein –2 Diacetate (DAF-2DA) is a non-fluorescent cell permeable reagent that can measure free Nitric Oxide (NO) and nitric oxide synthase (NOS) activity in living cells under physiological conditions. Once inside the cell the diacetate groups on the DAF-2DA reagent are hydrolyzed by cytosolic esterases thus releasing DAF-2 and sequestering the reagent inside the cell. Production of nitric oxide converts the non-fluorescent dye, DAF-2, to its fluorescent triazole derivative, DAF-2T.

DAF-2T can be observed by: excitation 488nm and measuring emission at 515nm.

### Reactivity

DAF-2DA can be used to detect NOS activity in cell culture or tissue sections. This reagent is not species specific and can also be used to detect NOS activity in plant cells.

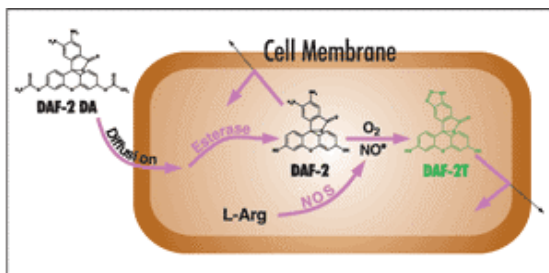
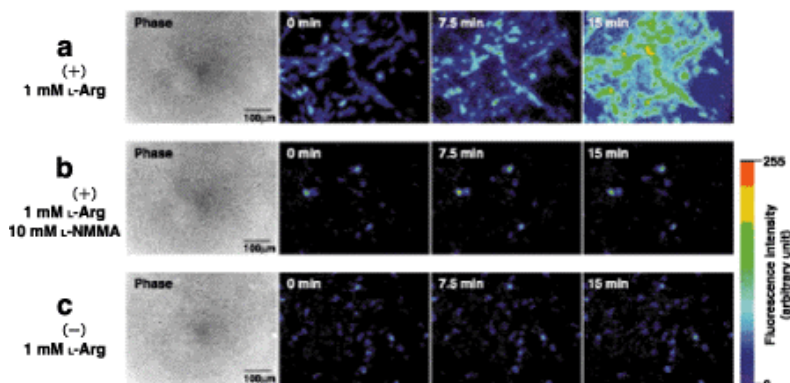


Fig 1. DAF-2DA, the non-fluorescent probe, enters the cells where cytosolic esterases hydrolyze the diacetate groups, releasing the non-fluorescent DAF-2 dye. DAF-2 then reacts with NO and O<sub>2</sub> to produce DAF-2T, its triazole fluorescent derivative.





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Fig 2. Phase Contrast and fluorescent microscopic images of rat aorta-derived vascular smooth muscular cells loaded with DAF-2DA.



## Product Specifications

Formula:  $C_{24}H_{18}N_2O_7$

MW: 446.4

Appearance: Liquid

Formulation: DAF-2DA is dissolved in DMSO at approx 5mM.

Manufactured: by Daiichi Pure Chemicals Co. Ltd. Japan

\*Not For Sale in Japan

## Product Catalog Information

1. NOS 200-1 ..... 1 vial .125 mg per vial at 2.22 mg/mL.
2. NOS 200-2.....1 vial .250mg per vial at 2.22 mg/mL

Dilute 1:250 to 1:500 for a 1X working solution.

## References.

1. *Direct evidence of NO production in rat hippocampus and cortex using a new fluorescent indicator: DAF-2 DA:* H. Kojima, et al.; Neuroreport **9**, 3345 (1998) Abstract
2. *Direct evidence of nitric oxide production from bovine aortic endothelial cells using new fluorescence indicators: diaminofluoresceins:* N. Nakatsubo, et al.; FEBS Lett. **427**, 263 (1998) Abstract
3. *Nitric Oxide Ameliorates Hydrophobic Bile Acid-induced Apoptosis in Isolated Rat Hepatocytes by Non-mitochondrial Pathways:* E. Gumpricht, et al.; J. Biol. Chem. **277**, 25823 (2002) Full Text;  
<http://www.jbc.org/cgi/content/full/277/28/25823#SEC1>
4. *Development of a fluorescent indicator for nitric oxide based on the fluorescein chromophore:* H. Kojima, et al.; Chem. Pharm. Bull. **46**, 373 (1998) Abstract
5. *Detection and imaging of nitric oxide with novel fluorescent indicators: diaminofluoresceins:* H. Kojima, et al.; Anal. Chem. **70**, 2446 (1998) Abstract

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