

# Diaminofluorescein-2 diacetate (DAF-2 DA)

Table 1. Product information

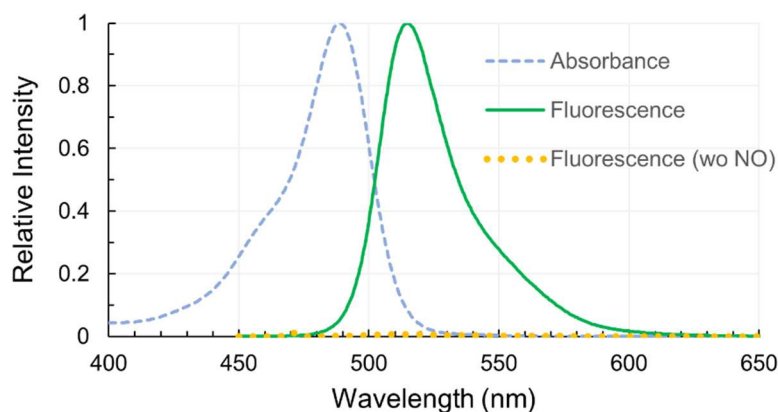
Catalog no.	Product name	Amount	Storage upon	Stability
SK1002-01	Diaminofluorescein-2 diacetate (DAF-2 DA)	1 mg (in DMSO 0.45 mL)	≤-20°C, keep desiccated and protected from light.	1 year (when unopened and stored as described.)

## 1. About DAF-2 DA

Diaminofluorescein-2 diacetate (DAF-2 DA) is a fluorescent probe to detect intracellular nitric oxide (NO). Cell permeable DAF-2 DA is hydrolyzed by intracellular esterase to generate cell impermeable DAF-2, which fluoresces upon reaction with NO. Therefore it is suitable for imaging of intracellular NO.

## 2. Properties of the reagent

The reagent is provided as a solution. It has almost no fluorescence but shows green fluorescence upon reaction with NO, with maximum emission at 515 nm. Optimum emission at 515 nm. Optimum excitation wavelength is 488 nm. For the observation with fluorescence microscopy, blue excitation filter set such as that for GFP and FITC is appropriate.



## 3. Preparation of the reagent

The reagent is a solution of 5 mM. To

avoid moisture absorption, allow the vial to reach room temperature before opening the cap. Dilute the solution 500-fold with neutral buffer solution such as PBS (pH=7.4) to prepare working solution of final concentration 10 μM. The concentration of the reagents should be optimized depending on the purpose of the experiment and the NO concentration to be detected.

## 4. Precautions

- 1) Prepare working solutions just before use.
- 2) The quality of the reagent may be compromised after opening the cap. Avoid repeated freeze-thaw cycles which may damage the reagent.
- 3) Use neutral buffer (pH=7 to 7.5) to dilute the reagent. Addition of bovine serum albumin (BSA), phenol red, calcium ion and vitamins may affect the fluorescence.
- 4) The reagent is dissolved in dimethyl sulfoxide which is flammable. Handle with precautions.

## 5. References

1. Kojima, H., Sakurai, K., Kikuchi, K., Kawahara, S., Kirino Y., Nagoshi, H., Hirata Y., Nagano, T. (1998) *Chem. Pharm. Bull.*, **46**:373–375
2. Kojima, H., Nakatsubo, N., Kikuchi, K., Kawahara, S., Kirino, Y., Nagoshi, H., Hirata, Y., and Nagano, T. (1998) *Anal. Chem.*, **70**:2446–2453
3. Nakatsubo, N., Kojima, H., Kikuchi, K., Nagoshi, H., Hirata, Y., Maeda D, Imai Y, Irimura T and Nagano, T. (1998) *FEBS Letters*, **427**:263–266
4. Kojima, H., Nakatsubo, N., Kikuchi, K., Urano, Y., Higuchi, T., Tanaka, J., Kudo, Y., Nagano, T. (1998) *Neuroreport*, **9**:3345–3348

Table 2. Related Products

Catalog no.	Product name	Major applications
SK1001-01	DAF-2	Detection of NO via green fluorescence.
SK1003-01	DAF-FM	Detection of NO via green fluorescence (in pH $\geq$ 6)
SK1004-01	DAF-FM DA	Detection and imaging of intracellular NO in pH $\geq$ 6, via green fluorescence.
SK1005-01	DAR-4M	Detection of NO in pH range of 4-12 via orange fluorescence.
SK1006-01	DAR-4M AM	Detection of intracellular NO in pH range of 4-12 via orange fluorescence.