

## Technical Data Sheet

## DAF-FM DA

## Product Information

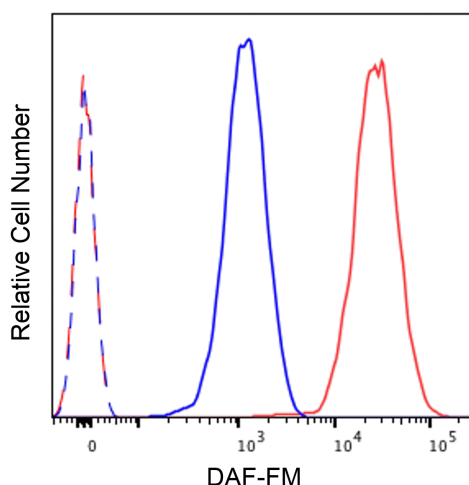
Material Number:	566663
Size:	500 µg
Component:	51-9015104
Description:	DAF-FM DA
Size:	50 µg (10 ea)

## Description

Nitric oxide is a signaling molecule involved in nervous system signal transduction, blood vessel tone in the cardiovascular system, and host defense in immunological systems. In these systems, nitric oxide is predominantly produced by the enzymes nNOS, eNOS, and iNOS, respectively. Because nitric oxide is a free radical, its overproduction is implicated in cytotoxicity in inflammatory disorders.

BD Pharmingen™ DAF-FM DA (Diaminofluorescein-FM Diacetate) provides an indicator to measure the levels of intracellular nitric oxide in live cells. In its unreacted form, the probe has minimal fluorescence and freely crosses the cell membrane. Once inside the cell, intracellular esterases cleave off the diacetate groups, trapping the now weakly fluorescent probe within the cell. The freed probe then reacts with nitric oxide to form a benzotriazole derivative which is highly fluorescent. Cells with higher levels of intracellular nitric oxide will therefore display increased fluorescence intensity.

The reacted BD Pharmingen™ DAF-FM probe is excited by the blue laser (eg, 488 nm), with an excitation maximum of 495 nm and an emission maximum of 515 nm.



**Flow cytometric analysis of the conversion of BD Pharmingen™ DAF-FM DA to fluorescent DAF-FM by nitric oxide in Jurkat Cells treated with DEA NONOate.** Jurkat cells in 1× DPBS were preincubated with (solid line) or without (dashed line) 10 µM BD Pharmingen™ DAF-FM DA for 30 minutes at 37°C and then treated with (red) or without (blue) 10 µM Diethylamine NONOate (Sigma Aldrich, Cat. No. D5431) for 30 minutes at 37°C. Cells were washed once and then analyzed by flow cytometry. Treatment with the nitric oxide donor Diethylamine NONOate results in an increase in the fluorescence of DAF-FM DA loaded cells. Histograms showing the levels of the nitric oxide-induced fluorescent benzotriazole derivative were derived from gated events with the light scattering characteristics of intact Jurkat cells. Flow cytometric analysis was performed using a BD LSRFortessa™ Cell Analyzer System.

## Application Notes

## Application

Flow cytometry

Tested During Development

## Recommended Assay Procedure:

## Preparation

Bring DAF-FM DA dye powder and anhydrous Dimethyl Sulfoxide (DMSO) to room temperature. Add 10 µL of DMSO to dye powder and vortex solution well. Inspect the solution and repeat vortex until the stock dye has fully dissolved. This yields a 10 mM stock solution.

## Storage

Upon arrival, store the dry dye with desiccant and protected from light at -20°C until use. We recommend a fresh vial of dye be used for each experiment and that reconstituted dye be discarded after use. However, if stock solutions in DMSO are to be kept for use, they should be stored with desiccant and protected from light at -20°C. Working solution that have been diluted into aqueous solution from DMSO should not be kept for further use.

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566663 Rev. 1



## **Cytometry Requirements**

Blue laser-equipped flow cytometers (eg, BD FACSCanto™ II, BD LSRFortessa™, BD LSR™ II, or BD Accuri™ C6) can be used. This dye can be read out of filters commonly used for FITC (eg, 530/30-nm bandpass filter). Fluorescence compensation is best achieved using stained and unstained samples of the target cells.

When designing multicolor panels, please be aware of spillover into the PE and BD Horizon™ PE-CF594 channels on the blue laser. If available, collecting fluorescent signals from these fluorochromes using the yellow-green (eg, 561 nm) laser may be advantageous to avoid spillover from DAF-FM fluorescence. Staining panels should be optimized to take this spillover into account. Additionally, for multicolor panels, we recommend using the lowest concentration of DAF-FM DA that still provides adequate resolution for the cell type and conditions of interest.

## **Procedure**

### *Staining of Live Cells for Analysis by Flow Cytometry*

1. Count cells to determine cell density. If necessary, adjust cell density to  $1 \times 10^6$  cells/mL in fresh, pre-warmed  $1 \times$  DPBS.
  - a. Buffers containing serum, BSA, or phenol red may affect the fluorescence of DAF-FM DA. Therefore, these buffers should be used with caution.
  - b. Esterase activity in serum can cleave AM moieties from the dye prior to entry into cells. Therefore, if serum must be used, it should be heat-inactivated.
2. Add dye stock solution for a final staining concentration of 1-10  $\mu$ M and vortex immediately.
  - a. We recommend titrating the dye for optimal performance, as different cell types, incubation periods, or culture conditions can result in variability in staining.
3. Incubate 15-60 minutes at 37°C.
4. Wash twice with buffer of choice and resuspend cells in analysis or treatment buffer of choice.
  - a. For some cell types with low esterase activity, it may be advantageous to incubate cells for another 30-60 minutes to allow complete de-esterification of AM moieties. In this case, cells should be incubated in a physiologic buffer of choice or complete medium, washed once more, and then resuspended in analysis or treatment buffer of choice.
5. Treat cells at 37°C for a desired period of time to generate nitric oxide.
  - a. A positive control can be generated by treating cells with 1 mM DEA NONOate (Sigma Cat. No. D5431) for 30-60 minutes at 37°C.
6. Wash cells once to remove treatment compounds.
7. Proceed to analysis by flow cytometry.

## **Product Notices**

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at [www.bdbiosciences.com/colors](http://www.bdbiosciences.com/colors).
3. Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
4. CF™ is a trademark of Biotium, Inc.
5. Please refer to [www.bdbiosciences.com/pharming/protocols](http://www.bdbiosciences.com/pharming/protocols) for technical protocols.

## **References**

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