Detection and Bioimaging of Nitric Oxide (NO) Using Multicolor DAX–J2™ Reagents

Introduction

DAF-2 reagents are frequently used to detect nitric oxide (NO). However, DAF-2 diacetate is spontaneously hydrolyzed in cell culture media. The hydrolyzed DAF-2 is not cell-permeable, thus causing high assay background. DAX-J2™ probes are developed as excellent replacements for DAF-2 for the detection and bioimaging of NO. Compared to DAF-2 reagents, DAX-J2™ reagents have longer wavelengths and better stability. AAT Bioquest offers three distinct DAX-J2™ multicolor imaging reagents for NO detection.

DAX-J2™ Red is a new nitric oxide (NO) sensor recently developed by AAT Bioquest. It is a non-fluorescent cell permeable reagent that can measure free NO and nitric oxide synthase (NOS) activity in living cells under physiological conditions. Once inside the cell, the blocking groups on the DAX-J2™ reagent are released to generate a highly red fluorescent product upon NO oxidation. The red fluorescent DAX-J2™ product can be detected using the filter set of Texas Red® that is equipped with most of flow cytometers and fluorescence microscopes.

DAX-J2™ Orange generates a bright orange fluorescent product that has spectra properties similar to those of Cy3® and TRITC. As DAX-J2™ Red, DAX-J2™ Orange is cell permeable, and can be used for measuring free NO and nitric oxide synthase (NOS) activity in living cells under physiological conditions. DAX-J2™ Orange can be readily loaded into live cells, and its fluorescence signal can be conveniently monitored using the filter set of Cy3® and TRITC.

DAX-J2™ IR is a new fluorogenic NO sensor that has near infrared fluorescence. In contrast with DAX-J2™ Red and Orange, this DAX-J2™ reagent is highly water-soluble and cell-impermeable. It enables NO detection *in vivo* using IVIS® Imaging System (Caliper) or Kodak Image Station.

DAX-J2™ Ratio 580/460 is a newest nitric oxide (NO) sensor recently developed by AAT Bioquest. It is a cell permeable reagent that can measure free NO and nitric oxide synthase (NOS) activity in living cells in a ratiometric mode under physiological conditions. Once inside the cell the blocking groups on the DAX-J2 reagent are released to induce fluorescence ratio changes at wavelengths of 580 and 460 nm upon NO oxidation. The fluorescence intensities at 580 nm and 460 nm can be detected using the filter sets of Cy3®/TRITC and BD Horizon™ V450/Pacific Blue. These two filter sets are equipped with most of flow cytometers and fluorescence microscope. DAX-J2 Ratio 580/460 has distinct advantages for NO detection than the popular DAF-2 NO probe: 1). It does not require esterase activity for NO detection. DAF-2 requires intracellular esterases to cleave its acetate groups for detecting NO activity. This esterase dependence often complicates the NO detection since esterse activities are affected by cell health and many other factors. 2). DAX-J2 product exhibits pH-independent fluorescence while DAF-2 has its fluorescence highly affected by pH. 3). DAX-J2 Ratio 580/460 can be monitored in a ratiometric mode.

Key Features of DAX-J2™ Nitric Oxide (NO) Detection Reagents

- No esterase activity required for NO detection.
- pH-independent spectral properties.
- Much better photostability compare to DAF-2.
- More tolerant to cell medium hydrolysis than DAF-2.
- Compatible with GFP cell lines or the applications that use FITC channel for multicolor cell analysis.

Chemical and Physical Properties

Catalog #	DAX-J2™ Dyes	Cell Permeability	Molecular Weight	Solvent	Excitation	Emission
16300	DAX-J2™ Orange	Yes	476.57	DMSO	545 nm	576 nm
16301	DAX-J2™ Red	Yes	608.77	DMSO	588 nm	610 nm
16302	DAX-J2™ IR	NO	1016.05	DMSO	780 nm	800 nm
16310	DAX-J2™ Ratio 580/460	Yes	830.97	DMSO	420/540 nm	460/580 nm

Note: The excitation (Ex) and Emisson (Em) wavelength in the above table are the chemical properties of the dyes. It is highly recommended to consult your instrument company with the proper Ex/Em wavelengths /filters to use. For example, when using a Gemini or FlexStaion fluorescence microplate reader (Molecular Devices), one will need to use Ex/Em = 540/590 nm (cut off at 570 nm) for cat#16300, and ratio of Ex/Em = 540/590 nm (cut off at 570 nm) vs. Ex/Em = 420/480 nm (cut off at 455 nm) for cat#16310 respectively.

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Technical Support: support@aatbio.com; Tel: 408-733-1055

Storage and Handling Conditions

Upon receipt DAX-J2™ dyes should be stored at -20°C, desiccated and protected from light.

Assay Protocol with DAX-J2™ Dyes

The following protocol only provides a guideline, and should be modified according to your specific needs. Treat cells as desired before making the DAX-J2™ working solution.

1) Prepare a 5 to 10 mM DAX-J2™ stock solution in DMSO. Make 1 to 10 µM working solution by diluting the DMSO stock solution into Hanks solution with 20 mM Hepes buffer (HHBS).
2) Treat cells as desired.
3) Incubate the cells with 1-10 µM (from Step #1) DAX-J2™ orange (cat#16300), Red (cat# 16301) or Ratio 580/460 (16310) for 20 -60 minutes at 37 ºC.
4) Replace the dye-loading solution with HHBS buffer.
5) Analyze the cells with a proper fluorescence instrument with bottom read mode.

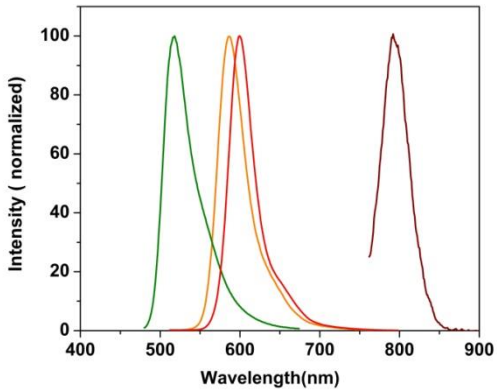

Figure 1. The emission spectra of DAX-J2™ reagents. DAF-2 (Green), DAX-J2™ Orange (Orange), Red (Red) and IR (Dark Red) in PBS buffer (pH 7.2).

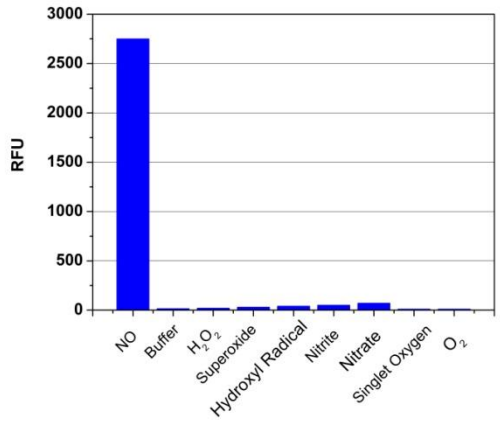

Figure 2. Fluorescence response of DAX-J2™ Orange (5 µM) to different reactive oxygen species (1 mM) in PBS buffer (pH 7.2). The fluorescence intensities were measured with Ex/Em = 540/590 nm (cut off at 570 nm) with a Gemini fluorescence microplate reader (Molecular Devices).

Disclaimer: *This product is for research use only and is not intended for therapeutic or diagnostic applications.*