Accelerating Scientific Discovery

Elite™ Extracellular Nitric Oxide Activity Assay Kit

Catalog Number: **CA-NO120** | Size: 1**00 assays**

1. Description

Nitric oxide (NO) is a short-lived signaling molecule involved in vascular tone, immune defense, neurotransmission, inflammation, and many other physiological and pathological processes. Because NO is rapidly oxidized and is generally present at low concentrations in biological systems, sensitive methods are required for reliable measurement.

The Elite™ Fluorimetric Extracellular Nitric Oxide (NO) Activity Assay Kit provides a rapid and sensitive method for monitoring NO in extracellular media, tissues, and other biological samples. The kit uses DAW-J2, a cell-impermeant fluorescent NO sensor. The nonfluorescent probe reacts with extracellular NO to generate a strongly red-fluorescent product that can be measured with a fluorescence microplate reader at Ex/Em = 570/610 nm.

2. Key Advantages

- Selective measurement of extracellular nitric oxide using a cell-impermeant probe
- Red fluorescence with reduced interference from common blue- and green-fluorescent sample components
- Simple mix-and-read workflow completed in 30–60 minutes
- Compatible with 96- and 384-well microplates
- Suitable for extracellular media, tissue preparations, and other biological solutions
- Fluorescence detection at Ex/Em = 570/610 nm

3. Kit Components

Component	Quantity / Format
Component A: DAW-J2	1 vial
Component B: Assay Buffer	1 bottle (10 mL)
Component C: DMSO	1 vial (50 µL)

4. Storage

Store all kit components frozen at or below −20 °C. Minimize exposure of DAW-J2 to light. After preparation, divide unused stock solutions into single-use aliquots and store at −20 °C. Avoid repeated freeze-thaw cycles.

5. Materials Required but Not Supplied

- Solid black 96-well microplate or 384-well equivalent
- Fluorescence microplate reader capable of Ex/Em = 570/610 nm (590 nm cutoff recommended)
- DEA NONOate (Cayman Chemical, Item No. 82100, CAS#372965-00-9) or another suitable nitric oxide donor for preparation of the standard curve
- 0.01 N NaOH for preparation of the nitric oxide donor stock solution

6. Assay Protocol (for One 96-Well Plate)

Add 50 µL Nitric oxide standards or Samples ▶ Add 50 µL DAW-J2 working solution ▶ Incubate at RT for 30-60 minutes ▶ Read fluorescence (Ex/Em 570/610 nm)

Thaw one vial or bottle of each required kit component at room temperature before beginning the assay. Protect DAW-J2 solutions from direct light.

Step 1. DAW-J2 Stock Solution (200X)

Add 25 µL of DMSO (Component C) to the vial of DAW-J2 (Component A). Mix gently until completely dissolved.

Step 2. Nitric Oxide Standard Stock Solution (100 mM; not supplied)

Prepare a 100 mM DEA NONOate stock solution in 0.01 N NaOH. DEA NONOate was used to generate the representative standard curve described in this protocol.

Note: Nitric oxide donor solutions are unstable after dilution. Prepare standards immediately before use and proceed promptly with the assay

Step 3. Nitric Oxide Standard Dilution

Add 10 µL of the 100 mM DEA NONOate stock solution to 990 µL of Assay Buffer (Component B) to prepare a 1 mM standard (SD7). Perform six consecutive 1:2 serial dilutions in Assay Buffer to prepare SD6 through SD1: 500, 250, 125, 62.5, 31.3, and 15.6 µM.

Step 4. DAW-J2 Working Solution (1X)

Add 25 µL of the 200X DAW-J2 stock solution to 5 mL of Assay Buffer (Component B) and mix well. This volume is sufficient for approximately 100 assays.

Note: Prepare the DAW-J2 working solution fresh for each experiment. Use promptly and protect from light

Step 5. Sample Experimental Assay

Table 1. Suggested Plate Layout

BL	BL	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS
SD1	SD1	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS
SD2	SD2	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS
SD3	SD3	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS
SD4	SD4	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS
SD5	SD5	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS
SD6	SD6	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS
SD7	SD7	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS

BL = Blank Control; SD = Nitric Oxide Standard (SD1-SD7); TS = Test Sample

Table 2. Reagent Addition Guide (each Well)

Well Type	Reagent	Volume
SD1–SD7	Nitric oxide standard dilution (15.6 to 1000 µM)	50 µL
BL	Assay Buffer	50 µL
TS	Test Sample	50 µL

Step 6. Run Assay

6.1 Add 50 µL of each standard, blank, or test sample to the appropriate wells of a solid black 96-well microplate.

6.2 Add 50 µL of freshly prepared DAW-J2 working solution to each well for a final assay volume of 100 µL per well.

6.3 Incubate for 30–60 minutes at room temperature, protected from light.

6.4 Measure fluorescence at Ex/Em = 570/610 nm. A 590 nm cutoff is recommended when available.

7. Data Analysis

Use the fluorescence reading from the blank well as the background control. Subtract the mean blank value from all standard and sample readings. Plot the baseline-corrected fluorescence intensity against the corresponding nitric oxide standard concentrations and fit the data using an appropriate regression model. Calculate sample nitric oxide concentrations from the resulting standard-curve equation, applying any sample dilution factor.

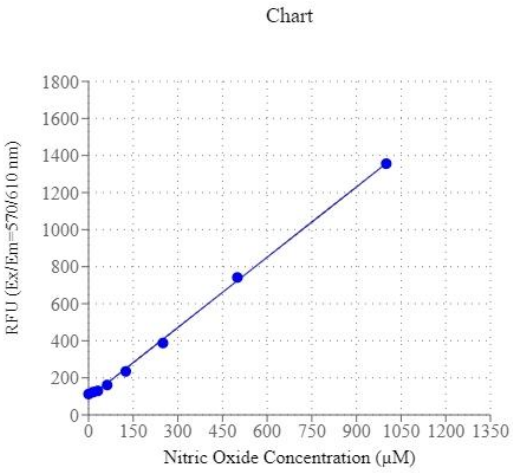

Figure 1. Representative nitric oxide dose response measured in a 96-well solid black microplate using fluorescence detection at Ex/Em = 570/610 nm. The plotted values are an approximate reconstruction of the representative curve shown in the supplied protocol and are provided for illustrative formatting purposes.