Accelerating Scientific Discovery

Elite™ Nitrite Quantification Assay Kit

Catalog Number: **CA-NO100** | Size: **100 assays**

1. Description

Nitric oxide (NO) is an important signaling molecule involved in vascular regulation, immune responses, neurotransmission, and inflammatory processes. Because NO is rapidly converted into nitrite and nitrate in biological systems, measurement of nitrite is widely used as a convenient indicator of nitric oxide production.

The Elite™ Nitrite Quantification Assay Kit provides a rapid colorimetric method for determining nitrite concentrations in biological samples using the Griess reaction. Under optimized assay conditions, nitrite reacts with the Griess reagent to generate a stable azo dye that can be quantified by measuring absorbance at 548 nm. Signal intensity is directly proportional to nitrite concentration.

2. Key Advantages

- Fast protocol: complete results in under 10 minutes
- Simple one-step colorimetric detection
- Compatible with serum, plasma, cell culture medium and other biological samples
- Linear detection range approximately 1–100 µM nitrite
- Suitable for 96- and 384-well microplates

3. Kit Components

Component	Quantity / Format
Component A: Griess Reagent	50 mL
Component B: Nitrite Standard (50 mM)	1 vial (25 µL)

4. Storage

Store all components at 2–8 °C. Protect Griess reagent from prolonged light exposure.

5. Materials Required but Not Supplied

- Clear 96-well microplates (or 384-well equivalent)
- Microplate reader capable of measuring absorbance at 548 nm

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

Add 75 µL Samples or Nitrite standards ▶ Add 75 µL Griess reagent working solution ▶ Incubate at RT for 10 minutes ▶ Monitor absorbance at 548 nm

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at **–20 °C** after preparation. Avoid repeated freeze-thaw cycles.

Step 1. Griess Reagent Working Solution

Add 1 mL of Griess Reagent (Component A) to 6.5 mL of distilled water. Mix well and protect from light.

Step 2. Nitrite Standard Solution (100 µM)

Add 4 µL of 50 mM nitrite standard solution (Component B) into 2 mL of distilled water to get 100 µM nitrite standard solution.

Step 3. Preparation of Nitrite Standard Series Dilutions - Nitrite Standard

Prepare a nitrite standard by diluting the 100 µM nitrite standard solution (Step 2) in distilled water using a dilution factor of 2, yielding a 7-point standard curve (SD1–SD7: 100, 50, 25, 12.5, 6.25, 3.13, 1.56 µM).

Step 4. 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 = Nitrite Standard (SD1-SD7); TS = Test Sample

Table 2. Reagent Addition Guide (each Well)

Well Type	Reagent	Volume
SD1–SD7	Trolox serial dilution (100 to 1.56 µM)	75 µL
BL	Distilled water	75 µL
TS	Test Sample	75 µL

Step 5. Run Assay

5.1. Add 75 µL standards or samples to each well.

5.2. Add 75 µL Griess reagent working solution.

5.3. Incubate 10 minutes at room temperature protected from light.

5.4. Measure absorbance at 548 nm.

7. Data Analysis

Subtract the blank absorbance from all readings. Generate a standard curve using nitrite standards and calculate sample concentrations by interpolation. Report results as µM nitrite.

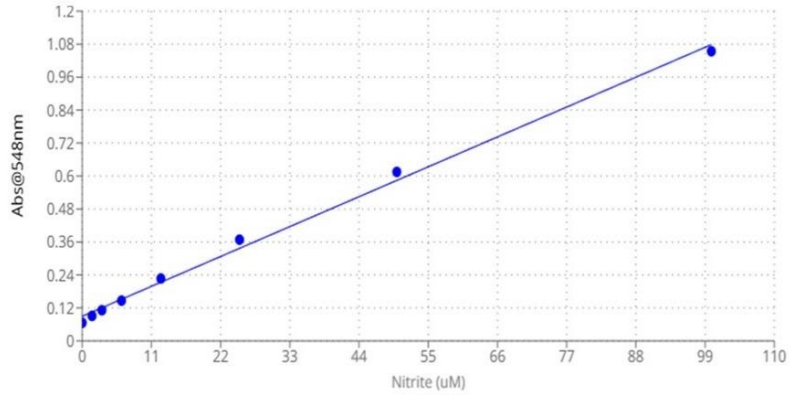

Figure 1. Nitrite dose response was measured with the Nitrite Quantification Assay Kit on a 96-well white microplate using a Clariostar microplate reader (BGM) at Absorbance 548nm.