EZ-Nitric Oxide Assay Kit

Metabolism assay kit

(Colorimetric)

Cat. No. DG-NO500

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

DoGenBio Co., Ltd.

▪ Product Description

Nitric oxide (NO) is a radical compound generated during the conversion of L-arginine to citrulline by nitric oxide synthase (NOS). It plays a critical role in neurotransmission, blood vessel regulation, immune responses, and cell death. NO also influences macrophage-mediated cytotoxicity and is known to inhibit mitochondrial respiration and DNA synthesis.

The EZ-Nitric Oxide Assay Kit is based on the diazotization reaction (Griess method) and measures the NO concentration in the sample. This kit accurately quantifies nitrate (NO_3^-) levels by measuring nitrite (NO_2^-), a conversion product of nitrate present in tissues or cells. The reaction between sulfanilamide and nitrite, followed by the addition of *N*-(1-naphthyl)ethylenediamine, generates a product that can be used to quantify the total NO_3^- concentration.

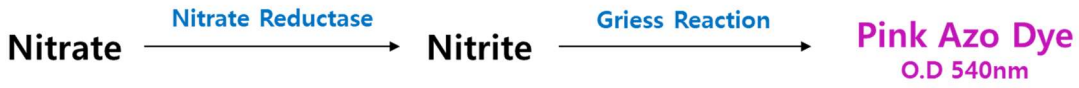

Fig 1. Nitrite quantification using the Griess reagent.

▪ Contents and Storage Conditions

Component	500 assay	Storage
Griess Reagent A	25 ㎖	4 ℃
Griess Reagent B	25 ㎖	
Nitrite Standard (2 mM)	500 ㎕ X 2 vial	

* It remains stable for 6 months at 4°C after opening.
* Do not mix Griess reagent A and B in advance; mix them just before use.

▪ General Protocol

Sample preparation

Note

• If the sample contains proteins, filter it using a 0.2 ㎛ filter before using it in the experiment.

- **Serum/Plasma**
  ① Use a filter to remove proteins from the sample.
  ② Centrifuge the sample at 10,000 × *g* for 10 minutes at 4°C and use only the supernatant for analysis.

- **Urine**
  ① Dilute the sample at least 10 times using PBS, filter it, and use it immediately.
  ② For more accurate measurements, prepare samples at various concentrations using PBS.

- **Cell lysate**
  ① Homogenize 1 × 10^6 cells quickly with 100 ㎕ of ice-cold PBS.
  ② Incubate on ice for 10 minutes, then centrifuge at 10,000 × *g* for 5 minutes at 4°C. Transfer the supernatant to a new tube.
  ③ Add 10–100 ㎕ of the sample to each well, then add PBS to adjust the total volume to 100 ㎕ for use in the experiment.

- **Culture supernatant**
  ① Avoid using media that contain nitrite.
  ② Transfer 100 ㎕ of the culture supernatant (without filtration) to a 96-well plate, and use it directly as the sample for analysis.
  ③ When using media with a strong color, dilute the nitrite standard solution in the same medium and prepare the standard curve.

Nitrite standard preparation

: Prepare the nitrite standard solution by diluting 2 mM nitrite solution and D.W as follows.

Standard Tube No.	Nitrite standard solution	D.W	Final standard Nitrite Conc. (μM)
1	50 ㎕ of 2 mM (2000 μM)	450 ㎕	200
2	250 ㎕ of Tube # 1	250 ㎕	100
3	250 ㎕ of Tube # 2	250 ㎕	50
4	250 ㎕ of Tube # 3	250 ㎕	25
5	250 ㎕ of Tube # 4	250 ㎕	12.5
6	250 ㎕ of Tube # 5	250 ㎕	6.25
7	250 ㎕ of Tube # 6	250 ㎕	3.125
8	0 ㎕	250 ㎕	0

* It is recommended that the standard be measured for each experiment.

* Prepare the Nitrite standard solution and sample in a volume of at least 250 ㎕ to allow for testing in duplicate or more for each concentration.

Nitric Oxide (Griess Reagent) Assay

① Before the experiment, store Griess reagents A and B at room temperature for about 15–30 minutes before use.

② Add 100 ㎕ of the Nitrite standard solution and sample into each well according to the concentration.

③ Add 50 ㎕ of Griess reagent A to each well and incubate in the dark at room temperature for 10 minutes.

④ Add 50 ㎕ of Griess reagent B to each well and incubate again in the dark at room temperature for 10 minutes.

⑤ After the reaction, measure the absorbance at 540 nm using a microplate reader. If a 540 nm wavelength is unavailable, measure within the range of 520–550 nm.

▪ Calculation

Nitrite concentration of sample (μM)

1. Correct the O.D. values for all measurements by subtracting the blank's O.D. value.
   * Blank = $O.D_{540nm}$ from Nitrite standard No. 8 (0 μM Nitrite)
2. Plot the standard curve using the O.D. values (Y-axis) versus the concentrations of Nitrite standard (X-axis) to obtain the linear equation.
3. Calculate the Nitrite amount in the sample by substituting the sample's O.D. value into the standard curve.
   * If a background control has been set, subtract the background control measurement from the sample's measurement before calculating the Nitrite amount.
   * If the sample was diluted 2-fold, multiply the calculated Nitrite concentration (μM) by 2 to obtain the accurate Nitrite amount.

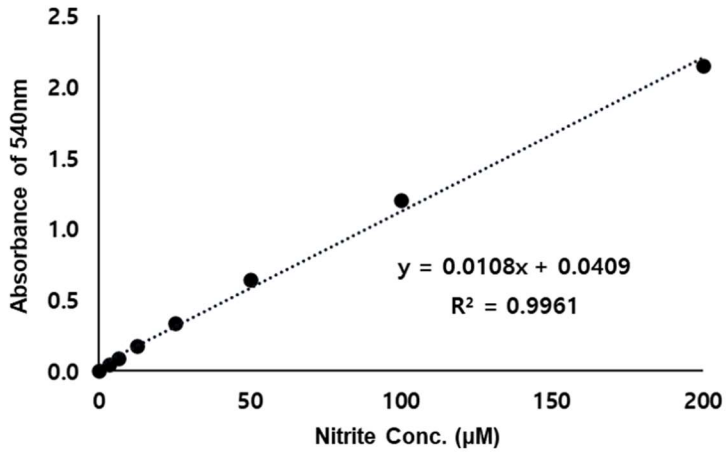

Fig 2. Nitrite standard curve. Assay was performed following the kit protocol.

▪ Related Product

	Products	Catalog No.	Assay
Oxidative Stress Assay Kit	EZ-Superoxide Dismutase (SOD) Assay Kit (Colorimetric)	DG-SOD400	400 Assay
	EZ-Glutathione Assay Kit (Colorimetric)	DG-GLU200	200 Assay
	EZ-Catalase Assay Kit (Fluorometric/Colorimetric)	DG-CAT400	400 Assay
	EZ-Hydrogen peroxide/Peroxidase Assay Kit (Fluorometric/Colorimetric)	DG-PER500	500 Assay
	EZ-Lipid Peroxidation (TBARS) Assay Kit (Colorimetric)	DG-TBA200	200 Assay
	EZ-Total Antioxidant Capacity (TAC) Assay Kit (Colorimetric)	DG-TAC200	200 Assay
	EZ-DPPH Antioxidant Assay Kit (Colorimetric)	DG-DPH400	400 Assay
Metabolism Assay Kit	EZ-Lactate Assay Kit (Colorimetric)	DG-LAC100	100 Assay
	EZ-Acetylcholinesterase Assay Kit (Colorimetric)	DG-ACE100	100 Assay
	EZ-Ascorbic Acid Assay Kit (Colorimetric)	DG-ASC100	100 Assay
	EZ-ATP Assay Kit (Fluorometric/Colorimetric)	DG-ATP100	100 Assay
	EZ-Free Fatty Acid Assay Kit (Fluorometric/Colorimetric)	DG-FFA100	100 Assay
	EZ-Free Glycerol Assay Kit (Fluorometric/Colorimetric)	DG-FGC100	100 Assay
	EZ-Glucose Assay Kit (Fluorometric/Colorimetric)	DG-GCS100	100 Assay
	EZ-HDL, LDL/VDL Assay Kit (Fluorometric/Colorimetric)	DG-CHO100	100 Assay
	EZ-Total Cholesterol Assay Kit (Fluorometric/Colorimetric)	DG-TSC100	100 Assay
	EZ-Triglyceride Quantification Assay Kit (Fluorometric/Colorimetric)	DG-TGC100	100 Assay