TECHNICAL MANUAL

Nitric Oxide (NO) Colorimetric Assay Kit

- **SKU CODE:** MAES0050
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

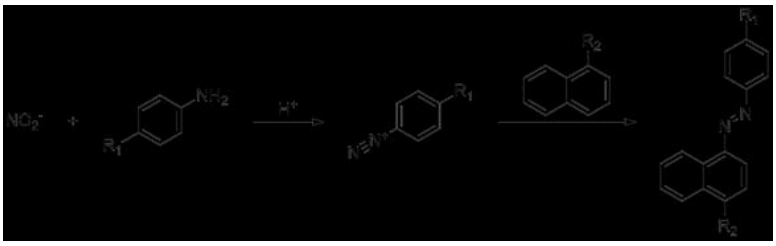
- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Chromogenic reaction
- Measure absorbance at 550 nm

2. Intended use

This kit can be used to measure nitric oxide (NO) in serum, plasma, saliva, and animal and plant tissue samples.

3. Detection principle

NO is easily oxidized to form NO_2^- in vivo or in aqueous solution, and a reddish azo compound is formed with the color developing agent. The concentration of the azo compound is linearly related to the concentration of NO. The concentration of NO can be calculated indirectly by measuring the absorbance at 550 nm.



4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Size3 (500 Assays)	Storage
Reagent 1	Sulphate Solution	12 mL × 1 vial	24 mL × 1 vial	24 mL × 5 vials	2-8°C, 12 months
Reagent 2	Alkali Reagent	12 mL × 1 vial	12 mL × 1 vial	12 mL × 5 vials	2-8°C, 12 months

Item	Component	Size 1(48 T)	Size 2(96 T)	Size3 (500 Assays)	Storage
Reagent 3	Chromogenic Agent A	1.9 mL × 1 vial	1.9 mL × 2 vials	1.9 mL × 10 vials	2-8°C, 12 months, protect from light
Reagent 4	Chromogenic Agent B	Powder × 1 vial	Powder × 1 vial	Powder × 5 vials	2-8°C, 12 months, protect from light
Reagent 5	Acid Solution	1.3 mL × 1 vial	1.3 mL × 2 vials	1.3 mL × 10 vials	2-8°C, 12 months
Reagent 6	Sodium Nitrite Standard	Powder × 1 vial	Powder × 2 vials	Powder × 10 vials	-20°C, 12 months
	Microplate	48 wells	96 wells	/	No requirement
	Plate Sealer	2 pieces			

5. Materials prepared by users

Instruments:

Microplate reader (540-550 nm), micropipettor, vortex mixer, centrifuge

Reagents:

Double distilled water, normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. If there is any crystal precipitation in chromogenic agent A, dissolve it completely using a water bath at above 60°C before use.
3. **Preparation of chromogenic agent B working solution:** Dissolve one vial of chromogenic agent B with 3.8 mL of double distilled water and mix well to dissolve. Store at 4°C for 2 months, protected from light.
4. **Preparation of chromogenic reagent:** For each well, prepare 80 µL of chromogenic reagent (mix 30 µL of chromogenic agent A, 30 µL of chromogenic agent B working solution, and 20 µL of acid solution). The chromogenic reagent should be prepared fresh and cannot be used when its color becomes darker.

5. **Preparation of 2 mmol/L sodium nitrite standard:** Dissolve one vial of sodium nitrite standard with 2 mL of double-distilled water. The 2 mmol/L sodium nitrite standard should be prepared fresh.
6. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 2 mmol/L sodium nitrite standard with double distilled water to create a serial dilution. The recommended dilution gradient is as follows: 0, 10, 20, 30, 40, 50, 60, 100 μmol/L.

7. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not analyzed on the same day, serum or plasma can be stored at -80°C for one month.

Tissue sample:

1. Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

2. Wash tissue in cold PBS (0.01 M, pH 7.4).

3. Homogenize 20 mg tissue in 180 μL PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4°C.

4. Centrifuge at 10,000 × g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample:

The recommended dilution factors for different samples are as follows (for reference only):

- Human serum: 1

- Human plasma: 1

- Rat serum: 1

- Rat plasma: 1

- 10% Mouse liver tissue homogenate: 1

- 10% Epipremnum aureum tissue homogenate: 1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. Use disposable EP tubes or clean glass tubes with stoppers for centrifugation.
2. The supernatant for assay should not contain sediment, otherwise it will affect the results.
3. All reagents should be prepared the day before the assay and allowed to dissolve fully. Add reagents to the bottom of wells vertically and slowly, avoiding adding on the wall of the well and generating bubbles.
4. Serum samples can be stored for 3 days at 4°C and for one month at -20°C.

9. Operating steps

1. **Standard tubes: Add a* µL of sodium nitrite standard solution with different concentrations to 1.5 mL EP tubes.:** Sample tubes: Add a* µL of sample to 1.5 mL EP tubes. [Note]: a* = Sample volume = Standard volume. For serum or plasma samples, a* is 200-300 µL. For tissue samples, a* is 100-300 µL.
2. Add 200 µL of sulphate solution and mix thoroughly with a vortex mixer.
3. Add 100 µL of alkali reagent and mix thoroughly with a vortex mixer.
4. Stand for 15 min at room temperature, then centrifuge at 3,100 × g for 10 min. (If there is precipitate in the supernatant, transfer the supernatant to a new EP tube and centrifuge again.)
5. Take 160 µL of supernatant to the corresponding wells of the microplate for chromogenic reaction.
6. Add 80 µL of chromogenic reagent to each well, oscillate for 2 min and stand at room temperature for 15 min.
7. Measure the absorbance at 550 nm with a microplate reader.

10. Calculation

The standard curve:

1. Average the duplicate readings for each standard.

2. Subtract the mean absorbance value of the blank (Standard #1) from all standard readings. This is the absolute absorbance value.

3. Plot the standard curve using the absolute absorbance value of standards and corresponding concentrations as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or Excel).

The sample:

1. Serum (plasma) sample:

NO content (μmol/L) = (ΔA_550 - b) ÷ a × f

2. Tissue:

NO content (μmol/g_{prot}) = (ΔA_550 - b) ÷ a × f ÷ C_{pr}

[Note]

ΔA_550: Absolute absorbance, A_{sample} - A_{blank}

f: Dilution factor of sample before test

C_{pr}: Concentration of protein in sample, mg_{prot}/mL

11. Appendix I Performance Characteristics

Parameters:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	1.30	36.50	74.20
%CV	2.8	2.1	2.3

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	1.30	36.50	74.20
%CV	3.5	3.8	3.8

Recovery

Three samples of high, middle, and low concentrations were tested in parallel 6 times for each concentration to obtain an average recovery rate of 102%.

Recovery

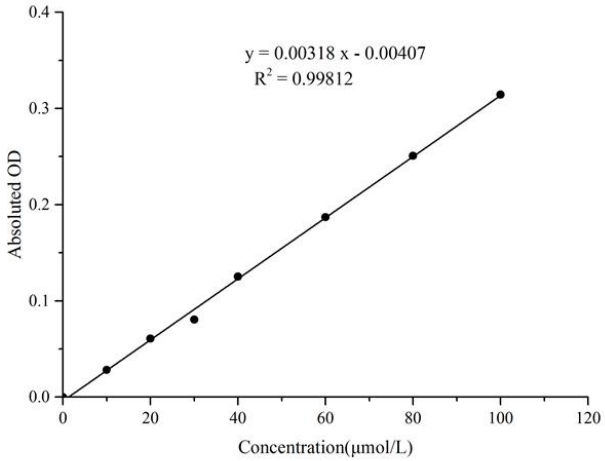
	Standard 1	Standard 2	Standard 3
Expected Conc. (µmol/L)	15	34	78
Observed Conc. (µmol/L)	15.2	35.4	78.8
Recovery rate (%)	101	104	101

Sensitivity

The analytical sensitivity of the assay is 0.16 µmol/L NO. This was determined by adding two standard deviations to the mean absorbance obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

As the absorbance value of the standard curve may vary according to the conditions of the actual assay performance (e.g., operator, pipetting technique, or temperature effects), the standard curve and data are provided below for reference only:



Standard curve

Concentration (μmol/L)	Average OD	Absolute OD
0	0.036	0.000
10	0.065	0.028
20	0.097	0.061
30	0.117	0.081
40	0.162	0.125
60	0.223	0.187
80	0.287	0.251
100	0.351	0.314

12. Appendix II Example Analysis

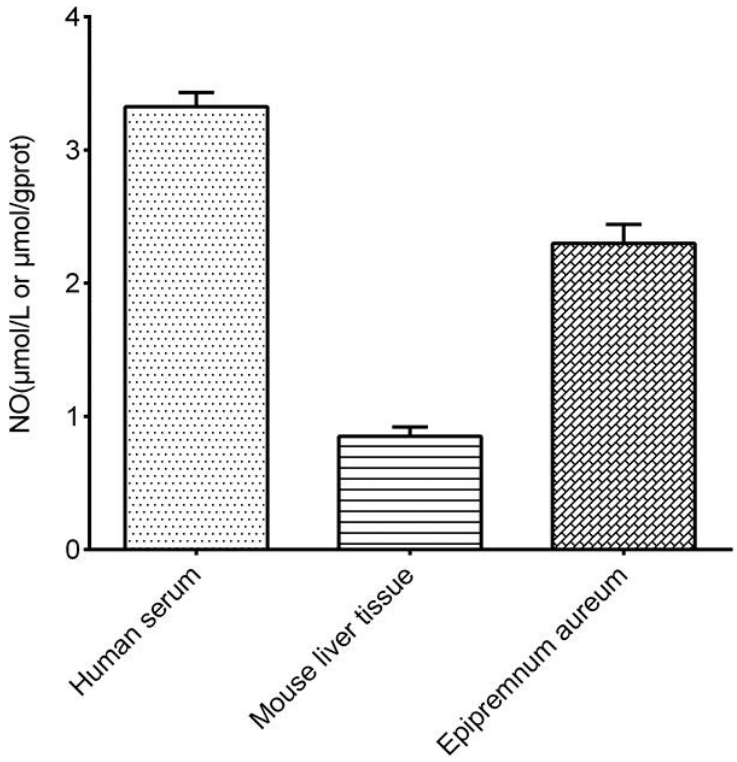
Example analysis:

Dilute human serum 2-fold and carry out the assay according to the operating steps. The results are as follows:

Standard curve: y = 0.00215x + 0.00514, the average absorbance value of the sample is 0.056, the average absorbance value of the blank is 0.035, the concentration of protein in sample is 9.23 g_{prot}/L, and the calculation result is:

NO content (μmol/L) = (0.056 - 0.035 - 0.00514) ÷ 0.00215 = 3.20 (μmol/L)

Detect human serum (diluted 2-fold, a* = 100 μL), 10% mouse liver tissue homogenate (protein concentration is 1.82 g_{prot}/L, a* = 300 μL), 5% Epipremnum aureum leaves tissue homogenate (protein concentration in sample is 0.99 g_{prot}/L, a* = 300 μL), according to the protocol. The results are as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.