TECHNICAL MANUAL

Nitric Oxide (NO) Colorimetric Assay Kit (Nitrate Reductase)

- **SKU CODE:** MAES0251
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Incubation reaction
- Chromogenic reaction
- Calculate results

2. Intended use

This kit can detect the concentration of nitric oxide (NO) in serum, plasma, urine, tissue, cell, and cell culture supernatant samples.

3. Detection principle

Nitric oxide (NO) has highly reactive chemical properties and is rapidly metabolized into NO2- and NO3- in the body, while NO2- is further converted to NO3-. This method uses nitrate reductase to specifically reduce NO3- to NO2-, and the concentration of NO is measured by the color intensity developed.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Enzyme Reagent	Powder x 2 vials	Powder x 4 vials	-20 °C, 12 months, shading light
Reagent 2	Substrate	Powder x 1 vial	Powder x 1 vial	-20 °C, 12 months, shading light
Reagent 3	Sulfate Solution	1.5 mL x 1 vial	3 mL x 1 vial	-20 °C, 12 months
Reagent 4	Alkaline Reagent	0.75 mL x 1 vial	1.5 mL x 1 vial	-20 °C, 12 months
Reagent 5	Chromogenic Agent A	Powder x 1 vial	Powder x 1 vial	-20 °C, 12 months, shading light

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 6	Chromogenic Agent B	Powder x 1 vial	Powder x 1 vial	-20 °C, 12 months, shading light
Reagent 7	Acid Reagent	1.5 mL x 1 vial	3 mL x 1 vial	-20 °C, 12 months
Reagent 8	1 mmol/L Standard	0.75 mL x 1 vial	1.5 mL x 2 vials	-20 °C, 12 months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		

5. Materials prepared by users

Instruments: Vortex mixer, 37 °C incubator, centrifuge, microplate reader (520-550 nm, optimum wavelength: 530 nm)

6. Reagent preparation

1. Keep enzyme reagent and substrate on ice during use. Equilibrate all reagents to room temperature before use.
2. **Preparation of enzyme stock solution:** Dissolve one vial of enzyme reagent with 1 mL of ultrapure water, mix well. Store at 2-8 °C for 6 hours protected from light.
3. **Preparation of substrate working solution:** Dissolve one vial of substrate with 5 mL of ultrapure water, mix well. Store at -20 °C for 3 days protected from light.
4. **Preparation of enzyme working solution:** For each well, prepare 60 µL of enzyme working solution (mix well 30 µL of enzyme stock solution and 30 µL of substrate working solution). The enzyme working solution should be prepared fresh. Store at 2-8 °C for 6 hours protected from light.
5. **Preparation of chromogenic A working solution:** Dissolve one vial of chromogenic A with 6 mL of ultrapure water, heat and dissolve in 90 °C water bath. The working solution can be stored at 2-8 °C with shading light for 3 days and should be heated to dissolve in 90 °C water bath when used again.
6. **Preparation of chromogenic B working solution:** Dissolve one vial of chromogenic B with 4 mL of ultrapure water, mix well to dissolve. Store at 2-8 °C for 3 days protected from light.
7. **Preparation of chromogenic working solution:** Before testing, prepare sufficient chromogenic working solution according to the number of test wells. For example,

prepare 90 µL of chromogenic working solution (mix well 50 µL of chromogenic A working solution, 20 µL of chromogenic B working solution and 20 µL of acid reagent). The chromogenic working solution should be prepared fresh and protected from light.

8. **Preparation of 40 µmol/L standard solution:** Dilute 168 µL of 1 mmol/L standard with 4032 µL of ultrapure water, mix well. The 40 µmol/L standard solution should be prepared fresh and protected from light.
9. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 40 µmol/L standard solution with ultrapure water to serial concentrations. The recommended dilution gradient is as follows: 0, 8, 16, 20, 24, 28, 32, 40 µmol/L.

7. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not detected on the same day, the 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 40 mg).

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

3. Homogenize 40 mg tissue in 360 µL normal saline (0.9% NaCl) with a dounce homogenizer at 4 °C.

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

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

Cell (adherent or suspension) samples:

1. Harvest the number of cells needed for each assay (initial recommendation 1 x 10^6 cells).

2. Wash cells with PBS (0.01 M, pH 7.4).

3. Homogenize 1 x 10^6 cells in 300 µL normal saline (0.9% NaCl) with an ultrasonic cell disruptor at 4 °C.

4. Centrifuge at 10,000 x g for 10 min 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 factor for different samples is as follows (for reference only):

Human serum: 1

Human urine: 20-30

Mouse serum: 1-3

Chicken plasma: 1

Rat urine: 20-30

10% Rat brain tissue homogenate: 1

10% Rat kidney tissue homogenate: 1

10% Vegetable leaf tissue homogenate: 1

1 x 10^6 Jurkat cell: 1

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

8. The key points of the assay

1. The prepared chromogenic working solution should be stored protected from light.
2. After adding the enzyme working solution and sample or standard to the EP tube, mix thoroughly.
3. Avoid the precipitation at the bottom of EP tube when taking the supernatant from the incubation reaction.

9. Operating steps

Incubation reaction

① Standard tube: Take a* μL of standard solution with different concentrations to 1.5 mL EP tubes.

Sample tube: Take a* μL of sample to 1.5 mL EP tubes.

[Note]: a* = Sample volume = Standard volume. For tissue and cell samples, a* is 100-200 μL. For serum or plasma, a* is 80-100 μL.

② Add 60 μL of enzyme working solution to each tube.

③ Mix thoroughly and incubate at 37 °C with shading light for 60 min.

④ Add 20 μL of sulfate solution to each tube.

⑤ Add 10 μL of alkaline reagent to each tube.

⑥ Mix thoroughly and stand at room temperature for 5 min, centrifuge at 10,000 x g for 10 min, then take the supernatant for detection.

2. Chromogenic reaction

① Standard well: Add 50 μL of chromogenic working solution to the corresponding wells.

Sample well: Add 50 μL of chromogenic working solution to the corresponding wells.

② Standard well: Add 120 μL of supernatant from standard tube to the corresponding wells.

Sample well: Add 120 μL of the supernatant from sample tube to the corresponding wells.

③ Mix thoroughly with microplate reader for 5 s and stand at room temperature for 5 min. Measure the OD value of each well at 530 nm with microplate reader.

10. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

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

3. Plot the standard curve by using absolute OD value of standard and corresponding concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

The sample:

1. Serum (plasma) and other liquid sample:

NO content (μmol/L) = (deltaA$_{530}$ - b) ÷ a x f

2. Tissue and cell sample:

NO content (μmol/g$_{prot}$) = (deltaA$_{530}$ - b) ÷ a x f ÷ C$_{pr}$

[Note]

deltaA$_{530}$: the absolute OD value of sample, deltaA$_{530}$ = OD$_{sample}$ - OD$_{blank}$

f: dilution factor of the sample before tested.

C$_{pr}$: the protein content of sample (g$_{prot}$/L).

11. Appendix I Performance Characteristics

Parameter:

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)	5.50	18.50	32.00
%CV	6.5	5.8	5.7

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)	5.50	18.50	32.00
%CV	8.3	7.9	7.8

Recovery

Three samples of high concentration, middle concentration and low concentration were tested with 6 replicates for each concentration to obtain an average recovery rate of 95%.

Recovery

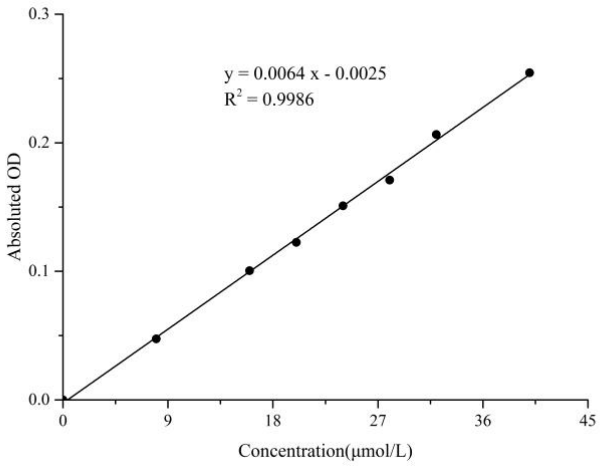
Standard	Expected Conc. (µmol/L)	Observed Conc. (µmol/L)	Recovery rate (%)
Standard 1	12	11.3	94
Standard 2	22.5	21.6	96
Standard 3	31	29.5	95

Sensitivity

The analytical sensitivity of the assay is 1.38 µmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve data

As the OD 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 data

Concentration (µmol/L)	OD value 1	OD value 2	Average OD	Absolute OD
0	0.043	0.043	0.043	0
8	0.092	0.089	0.091	0.048
16	0.144	0.143	0.144	0.101
20	0.166	0.165	0.166	0.123
24	0.194	0.194	0.194	0.151
28	0.214	0.214	0.214	0.171
32	0.249	0.250	0.250	0.207
40	0.295	0.300	0.298	0.255

12. Appendix II Example Analysis

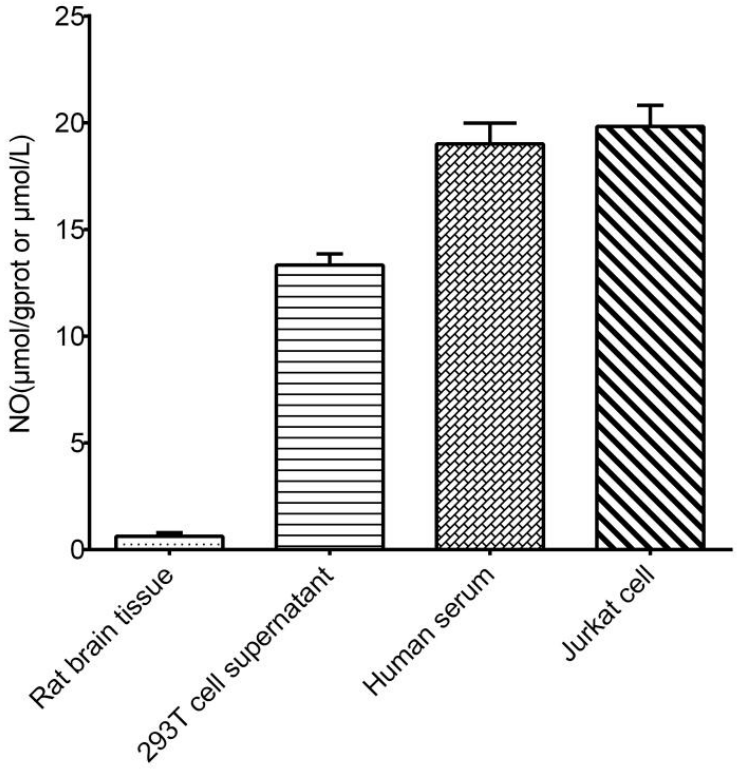
Example analysis:

For rat brain tissue, take 100 µL of 10% rat brain tissue homogenate, and perform the assay according to the operating steps. The results are as follows:

Standard curve: y = 0.0064x + 0.0025, the average OD value of the sample is 0.089, the average OD value of the blank is 0.060, the concentration of protein in sample is 4.74 g_{prot}/L, and the calculation result is:

NO content (µmol/g_{prot}) = (0.089 – 0.060 – 0.0025) ÷ 0.0064 ÷ 4.74 = 0.87 µmol/g_{prot}

Detect 10% rat brain tissue homogenate (the concentration of protein is 4.74 g_{prot}/L), 293T cell supernatant, human serum and Jurkat cell (the concentration of protein is 1.2 g_{prot}/L) 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.