（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

**Catalog No：E-BC-K035-S**

**Specification：50 Assays(48 samples)/ 100 Assays(96 samples)**

**Measuring instrument：Spectrophotometer (40-550 nm)**

**Detection range：0.97-700 μmol/L**

# **Elabscience®** Nitric Oxide (NO) Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623

Tell: 1-832-243-6086

Fax: 1-832-243-6017

Email: techsupport@elabscience.com

Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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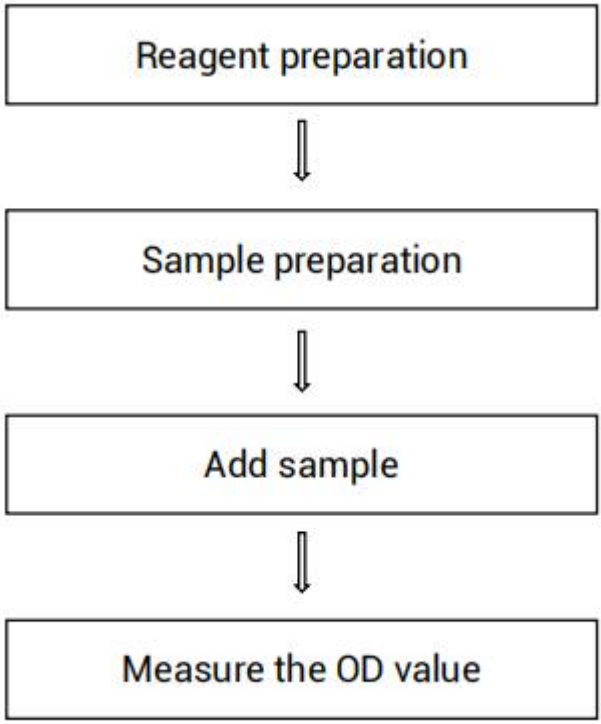
## Assay summary

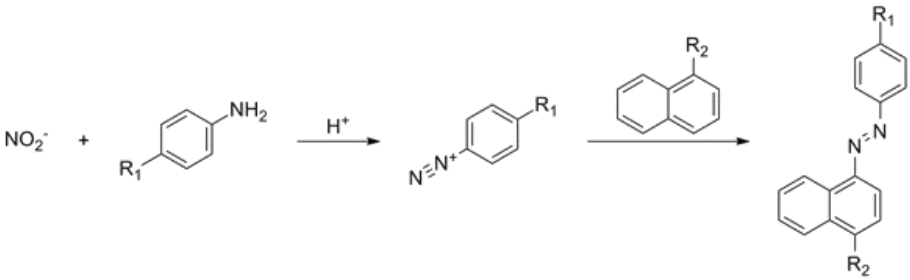


## Intended use

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

## Detection principle

NO is easily oxidized to form NO2- in vivo or in aqueous solution, and a reddish azo compound is formed with the color developing agent, and 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 OD value at 550 nm.




## Kit components & storage

| Item | Component | Size 1 (50 assays) | Size 2 (100 assays) | Storage |
|---|---|---|---|---|
| Reagent 1 | Sulphate Solution | 50 mL × 2 vials | 50 mL × 4 vials | 2-8℃, 12 months |
| Reagent 2 | Alkali Reagent | 50 mL × 1 vial | 50 mL × 2 vials | 2-8℃, 12 months |
| Reagent 3 | Chromogenic Agent A | 19 mL ×1 vial | 38 mL ×1 vial | 2-8℃, 12 months shading light |
| Reagent 4 | Chromogenic Agent B | Powder × 1 vial | Powder × 1 vial | 2-8℃, 12 months shading light |
| Reagent 5 | Acid Solution | 12.5 mL × 1 vial | 25 mL × 1 vial | 2-8℃, 12 months |
| Reagent 6 | Sodium Nitrite Standard | Powder × 1 vial | Powder ×2 vials | -20℃, 12 months |

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

## Materials prepared by users

**Instruments:**

Spectrophotometer (550 nm), Vortex mixer, Centrifuge, Analytical Balance, Micropipettor

**Reagents:**

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

## Reagent preparation

① Equilibrate other reagents to room temperature before use.

② If there is any crystal precipitation in chromogenic agent A, please dissolve it fully with water bath at above 60℃ before use.

③ The preparation of chromogenic agent B working solution：
Dissolve one vial of chromogenic agent B with 37.5 mL of double distilled water, mix well to dissolve. Store at 4°C for 2 month protected from light. If the reagents appear darkened color, it should be abandon. It is recommended to prepare the needed amount and the concentration is 1.5 mg/mL.

④ The preparation of chromogenic reagent：
For each tube, prepare 800 uL of chromogenic reagent (mix well 300 uL of chromogenic agent A, 300 uL of chromogenic agent B working solution and 200 uL of acid solution). The chromogenic reagent should be prepared on spot and can't be used when its color gets darker.

⑤ The preparation of 2 mmol/L standard solution：

Dissolve one vial of sodium nitrite standard with 2 mL of double-distilled water. The 2 mmol/L sodium nitrite standard should be prepared on spot.

⑥ The preparation of 40 μmol/L sodium nitrite standard solution：
Before testing, please prepare sufficient 40 μmol/L sodium nitrite standard solution according to the test wells. For example, prepare 300 uL of 40 μmol/L sodium nitrite standard solution (mix well 6 uL of 2 mmol/L standard solution and 294 uL of distilled water).

## Sample preparation

**① Sample preparation**

**Serum and plasma:** detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a 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℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

**② Dilution of sample**

The recommended dilution factor for different samples is as follows (for reference only):

| Sample type | Dilution factor |
|---|---|
| Human serum | 1 |
| Human plasma | 1 |
| Rat serum | 1 |
| Rat plasma | 1 |
| 10% Mouse liver tissue homogenization | 1 |
| 10% Epipremnum aureum tissue homogenization | 1 |

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

## The key points of the assay

① It is recommended to use a disposable plastic tube or glass tube must be washed clean.

② Hemolysis and turbid serum have an effect on the results of the experiment.

③ Serum samples can be stored for 3 days at 4℃ and for a month at -20℃.

④ The supernatant for chromogenic reaction should not contain sediment, otherwise it will affect the results.

## Operating steps

① Blank tube: add a* mL of double distilled water to 1.5 mL EP tubes.
Standard tubes: add a* μL of 40 μmol/L 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, a* is 100-300 μL.

② Add 1.6 mL of sulphate solution and mix fully with a vortex mixer.

③ Add 0.8 mL of alkali reagent and mix fully with a vortex mixer.

④ Stand for 15 min at room temperature, centrifuge at 3100 g for 10 min. (If there is precipitate in the supernatant, please transfer the supernatant to a new EP tube and centrifuge again.).

⑤ Take 1.6 mL of supernatant to the corresponding tubes for chromogenic reaction.

⑥ Add 0.8 mL of chromogenic reagent to each tube, mix well and stand at room temperature for 20 min.

⑦ Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 550 nm with 1 cm optical path cuvette.

## Calculation

**The sample**:

**1. Serum (plasma) sample:**

$$\text{NO content (μmol/L)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f$$

**2. Tissue:**

$$\text{NO content (μmol/gprot)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f \div C_{pr}$$

[Note]

$\Delta A_1$: $OD_{Sample} - OD_{Blank}$

$\Delta A_2$: $OD_{Standard} - OD_{Blank}$

c: Concentration of sodium nitrite, 40 μmol/L.

f: Dilution factor of sample before test.

$C_{pr}$: Concentration of protein in sample, gprot/L.

# Appendix I Performance Characteristics

## 1. Parameter:

### Intra-assay Precision

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

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| **Mean (μmol/L)** | 18.50 | 264.00 | 457.00 |
| **%CV** | 3.8 | 3.2 | 3.2 |

### Inter-assay Precision

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

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| **Mean (μmol/L)** | 18.50 | 264.00 | 457.00 |
| **%CV** | 5.0 | 4.9 | 5.7 |

### Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 99%.

| | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| **Expected Conc. (μmol/L)** | 164 | 395 | 522 |
| **Observed Conc. (μmol/L)** | 162.4 | 395.0 | 511.6 |
| **Recovery rate (%)** | 99 | 100 | 98 |

### Sensitivity

The analytical sensitivity of the assay is 0.97 μmol/L NO. 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.

## Appendix Π Example Analysis

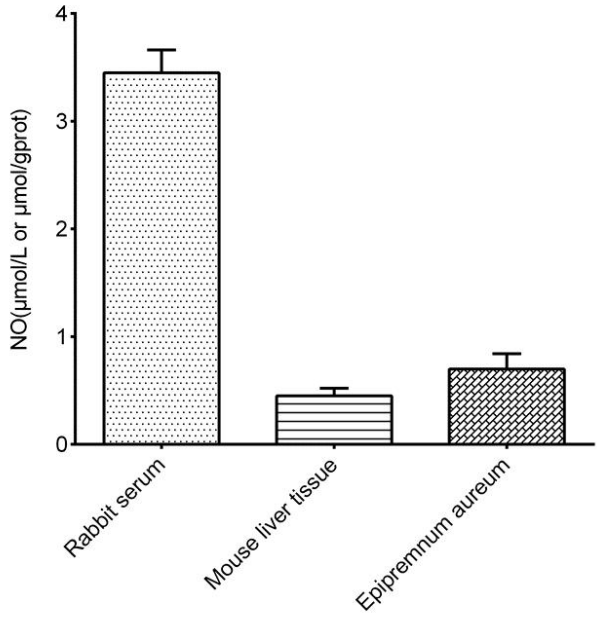
**Example analysis：**

Take 0.3 mL of 10% mouse liver tissue homogenate, carry the assay according to the operation steps. The results are as follows:

the average OD value of the sample is 0.010, the average OD value of the blank is 0.004, the average OD value of the standard is 0.065, the concentration of protein in sample is 8.65 gprot/L, and the calculation result is:

$$\text{NO content (μmol/gprot)} = \frac{0.010 - 0.004}{0.065 - 0.004} \times 40 \div 8.65 = 0.45 \text{ μmol/gprot}$$

Detect rabbit serum (a*=0.1 mL), 10% mouse liver tissue homogenate (the concentration of protein is 8.65 gprot/L, a*=0.3 mL), 10% Epipremnum aureum tissue homogenate (the concentration of protein in is 1.62 gprot/L, a*=0.4 mL), according to the protocol, the result is as follows：



# Appendix III Publications


1. Turhan D O, Güngördü A. Developmental, toxicological effects and recovery patterns in Xenopus laevis after exposure to penconazole-based fungicide during the metamorphosis process[J]. Chemosphere, 2022, 303: 135302.
2. Wang B, Liu J, Lei R, et al. Cold exposure, gut microbiota, and hypertension: A mechanistic study[J]. Science of the Total Environment, 2022, 833: 155199.
3. Yang H, Zhu Y, Ye Y, et al. Nitric oxide protects against cochlear hair cell damage and noise-induced hearing loss through glucose metabolic reprogramming[J]. Free Radical Biology and Medicine, 2022, 179: 229-241.
4. Pham D T, Thao N T P, Thuy B T P, et al. Silk fibroin hydrogel containing Sesbania sesban L. extract for rheumatoid arthritis treatment[J]. Drug delivery, 2022, 29(1): 882-888.
5. Syed R U, Hadi M A, Almarir A M, et al. Rhodiola rosea L. extract ameliorates ethanol-induced gastric ulcer in rats by alleviating oxidative stress and inflammation via NF-κB pathway inhibition[J]. Current Plant Biology, 2024, 40: 100421.
6. Popa A, Usatiuc L O, Scurtu I C, et al. Assessing the Anti-Inflammatory and Antioxidant Activity of Mangiferin in Murine Model for Myocarditis: Perspectives and Challenges[J]. International Journal of Molecular Sciences, 2024, 25(18): 9970.

## Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.