Nitric Oxide (NO) Assay Kit

Catalog No.: abx298829

Size: 96 tests

Detection Range: 0.16 µmol/L – 100 µmol/L

Sensitivity: 0.16 µmol/L

Storage: Store Sodium Nitrite Standard at -20°C for up to 12 months. Store all other components at 2-8°C for up to 12 months, Chromogenic Reagent A and Chromogenic Reagent B must be stored in the dark.

Application: For quantitative detection of Nitric Oxide concentrations in serum, plasma, tissue homogenates, and saliva.

Introduction:

Nitric oxide (NO, also referred to as nitrogen monoxide) is a colorless, sweet-smelling gas. The double bond between the nitrogen and oxygen atom results in unpaired valency; NO is a radical. This chemical is used by plants to kill pathogens and promote the growth of roots, while animals use it in vasodilation (hematophagous parasites sometimes administer extra NO to their hosts), as an intracellular messenger, and in the immune response. NO can cause oxidative stress to nearby cells, and reduced levels in plasma have been linked to hypertension.

Abbexa's Nitric Oxide (NO) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating NO concentrations. NO can be oxidized to form NO_2, which reacts with the components in this kit to produce a reddish azo compound. The concentration of the azo compound color is proportional to the concentration of NO, which can then be calculated indirectly by measuring the OD value of the azo compound at 550 nm.

Kit components

1. 96-well microplate
2. Sulphate Solution: 24 ml
3. Alkali Reagent: 12 ml
4. Chromogenic Reagent A: 2 × 1.9 ml
5. Chromogenic Reagent B: 1 vial
6. Acid solution: 2 × 1.3 ml
7. Sodium Nitrite Standard: 2 vials
8. Plate sealer: 2

Materials required but not provided

1. Microplate reader (540-550 nm)
2. Micropipette
3. Vortex mixer
4. Centrifuge
5. Double distilled water
6. Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum** and **Plasma:** Serum and plasma samples should be collected by conventional methods and can be tested directly. If not detected on the same day of collection, the serum or plasma can be stored at 4°C for up to 3 days or stored at between -20°C and -80°C for up to a month.
- **Saliva:** Gargle clean water and collect saliva sample after 30 minutes. Centrifuge at 10,000 × g at 4°C for 5 minutes. Then, take the supernatant, keep on ice, and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Tissue Homogenates:** Carefully weigh at least 20 mg of the tissue homogenate and wash in cold PBS (0.01 M, pH 7.4). For each 100 mg of homogenate, add 0.9 ml of PBS. Homogenize by hand, using a mechanical homogenizer, or by ultrasonication in PBS (0.01 M, pH 7.4). Centrifuge the homogenate at 10,000 x g at 4°C for 10 minutes. Collect the supernatant and assay immediately. The protein concentration in the supernatant should be determined separately (abx097193).

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4), then carry out the assay procedure. 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:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

Equilibriate all reagents to room temperature before use.

- **Chromogenic Reagent A Solution**
  If crystals are observed in Chromogenic Agent A, please fully dissolve with a water bath at above 60°C before use.

- **Chromogenic Reagent B Solution**
  Add 3.8 ml of double distilled water to one vial of Chromogenic Agent B to prepare Chromogenic Reagent B Solution. Mix thoroughly to ensure that Chromogenic Agent B is fully dissolved. Unused Chromogenic Reagent B Solution can be stored at 4°C for up to 2 months in the dark.

- **Chromogenic Reagent Working Solution**
  For each well, prepare Chromogenic Reagent Working Solution by thoroughly mixing Chromogenic Agent A, Chromogenic Agent B, and Acid Solution, in a 3:3:2 ratio. For example, to make 80 µl of Chromogenic Reagent Working Solution, thoroughly mix 30 µl of Chromogenic Agent A Solution, 30 µl of Chromogenic Reagent B Solution and 20 µl of Acid Solution). The Chromogenic Reagent Working Solution should be prepared immediately and can't be used when it's color darkens.

- **2 mmol/L Sodium Nitrite Standard**
  Add 2 ml of double-distilled water to one vial of Sodium Nitrite Standard to prepare 2 mmol/L Sodium Nitrite Standard. The 2 mmol/L Sodium Nitrite Standard should be prepared immediately, before use in the assay.

Standards: Label 7 tubes with 10 µmol/L, 20 µmol/L, 30 µmol/L, 40 µmol/L, 50 µmol/L, 60 µmol/L, and 100 µmol/L. Add 10 µl, 20 µl, 30 µl, 40 µl, 60 µl, 80 µl, and 100 µl of Standard (2 nmol/L) to the 10 µmol/L, 20 µmol/L, 30 µmol/L, 40 µmol/L, 50 µmol/L, 60 µmol/L, and 100 µmol/L tubes respectively, followed by 1990 µl, 1980 µl, 1970 µl, 1960 µl, 1940 µl, 1920 µl, and 1900 µl of double-distilled water, to prepare Standard Dilutions with concentrations 10 µmol/L, 20 µmol/L, 30 µmol/L, 40 µmol/L, 50 µmol/L, 60 µmol/L, and 100 µmol/L. These volumes are summarized in the following table:

Standard Dilution (µmol/L)	**10**	**20**	**30**	**40**	**50**	**60**	**100**
2 mmol/L Sodium Nitrite Standard (µl)	10	20	30	40	60	80	100
Double-distilled water (µl)	1990	1980	1970	1960	1940	1920	1900

For the blank, or 0 mg/ml standard, use double-distilled water. The volume of each standard will be 2000 µl.

B. Assay Procedure

1. Mark microcentrifuge tubes for each standard and sample. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 200-300 µl of serum or plasma sample; or 100-300 µl of tissue sample to each sample tube and control tube.
3. Add 200 µl of Sulfate Solution to all tubes and thoroughly mix with a vortex mixer.
4. Add 100 µl of Alkali Reagent to all tubes and thoroughly mix with a vortex mixer.
5. Allow to stand for 15 minutes at room temperature, then centrifuge at 3100 × g for 10 minutes. (If a precipitate is observed in the supernatant, transfer the supernatant to a new EP tube and centrifuge again).
6. Set the standard and sample wells on the microplate and record their positions.
7. Transfer 160 µl of supernatant to the corresponding wells of the microplate for the chromogenic reaction.
8. Add 80 µl of Chromogenic Reagent to each well, then tap the plate or use an orbital shaker to mix thoroughly for 2 minutes and leave to stand at room temperature for 15 minutes.
9. Measure the OD of each well with a microplate reader at 550 nm.

Note:

- Use disposable EP tubes or clean glass tubes with a stopper for centrifugation.
- The supernatant for the assay should not contain sediment, otherwise it will interfere with the results.
- Please add the reagents to the bottom of the well vertically and slowly. This avoids a bubble forming if adding the reagents on the wall of the well.

C. Calculation of Results

Plot the standard curve, using the OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the concentration of Nitric Oxide in each sample well can be derived with the following formulae:

1. Serum, Plasma and Saliva samples:

$$\text{Nitric Oxide (µmol/L)} = \mathrm{f} \times \frac{\Delta \mathbf{A_{550}} - \boldsymbol{b}}{\mathbf{a}}$$

2. Tissue samples:

$$\text{Nitric Oxide (µmol/}g\text{ Protein)} = \mathrm{f} \times \frac{\Delta \mathbf{A_{550}} - \boldsymbol{b}}{\mathbf{a} \times \mathbf{C_{Protein}}}$$

where:

$\Delta \mathbf{A_{550}}$	$OD_{Sample} - OD_{Blank}$.
f	Dilution factor of sample before test.
$\mathbf{C_{Protein}}$	Concentration of protein in sample (mg protein/ml).

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.