Nitric Oxide Assay Kit

Catalog No.: abx294028

Size: 100 tests

Detection Range: 0.97 µmol/L – 700 µmol/L

Sensitivity: 0.97 µmol/L

Storage: Store all components at 4°C in the dark.

Application: For detection and quantification of Nitric Oxide in serum, plasma, saliva, and animal/plant tissue homogenates.

Introduction

Abbexa's Nitric Oxide Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Nitric Oxide activity. Nitric Oxide is readily oxidized to NO_2^-, which reacts to form a red azo compound with an absorbance maximum at 550 nm. The intensity of the color is proportional to Nitric Oxide concentration, which can then be calculated from absorbance values.

Kit components

1. Sulfate Solution: 4 × 50 ml
2. Alkali Reagent: 2 × 50 ml
3. Detection Reagent A: 38 ml
4. Detection Reagent B: 1 × vial
5. Acid Solution: 25 ml
6. Sodium Nitrite Standard: 2 × vials

Materials required but not provided

1. Spectrophotometer (550 nm, 1 cm optical path cuvette)
2. Pipette and pipette tips
3. Analytical Balance
4. Centrifuge
5. Vortex mixer
6. Double Distilled Water
7. Normal Saline (0.9 % NaCl)
8. 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:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 25°C for 30 mins. Centrifuge at approximately 2000 × g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Plasma:** Collect plasma using heparin as the anticoagulant. Centrifuge for 10 mins at 700-1000 × g at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets), keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Tissue Homogenates:** Wash 0.02 g – 1 g of tissue with PBS. Add double distilled water to a ratio of 9:1 and homogenize manually, using a mechanical homogenizer or by ultrasonication, in an ice water bath. Collect the tissue homogenate, and stand at room temperature for 15 minutes, shaking every 5 minutes. Centrifuge at 10,000 × g at 4°C for 10 minutes, then carefully remove the supernatant. The protein concentration in the supernatant should be determined separately **(abx097193)**. Non-homogenized tissue samples may be stored safely at -80 ºC for up to one month.

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 distilled water, Normal Saline, or PBS, 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
10 % Mouse Liver tissue homogenate	1
Rat Serum	1
Rat Plasma	1
10 % *Epipremnum aureum* tissue homogenate	1

Notes:

- 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 assay. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Detection Reagent A:** May crystallize in storage, redissolve in a 60 ºC water bath if required.
- **Detection Reagent B:** Dissolve each vial in 37.5 ml double distilled water. The prepared solution may be stored at 4 ºC in the dark for up to 2 months. Do not use if the prepared solution exhibits a dark color.
- **Detection Reagent Working Solution:** Prepare a solution of Reagents A, B, and Acid Solution at a ratio of 3:3:2. Use immediately, discard if color darkens.
- **Standard Solution:** Dissolve each vial in 2 ml of double distilled water, prepare fresh before use.
- **Sodium Nitrite Solution (40 :** Dilute with double distilled water at a 1:49 ratio.

Note: Allow all reagents to equilibrate to room temperature before use.

B. Assay Procedure

1. Label Blank, Standard, and Sample tubes.
2. Add 0.2 ml double distilled water to each blank tube.
3. Add 0.2 ml of Sodium Nitrite solution to each standard tube.
4. Add 0.2 ml of sample to each sample tube.
5. Add 1.6 ml Sulfate Solution and mix thoroughly.
6. Add 0.8 ml Alkali Reagent and mix thoroughly.
7. Incubate at room temperature for 15 minutes, then centrifuge at 3100 × g for 10 minutes.
8. Transfer 1.6 ml supernatant to fresh tubes and add 0.8 ml Detection Reagent Working Solution, mix thoroughly, then incubate at room temperature for 20 minutes.
9. Set the spectrophotometer to zero with double distilled water at 550 nm.
10. Measure OD values in spectrophotometer at 550 nm using 1 cm optical path cuvettes.

C. Calculation of Results

The concentration of Nitric Oxide in each sample can be derived with the following formulae:

1. Serum, Plasma, and Saliva samples:

$$\textbf{NO concentration } (\textbf{µmol/L}) = \frac{\Delta \boldsymbol{A_1} \times \boldsymbol{c}}{\Delta \boldsymbol{A_2}} \times \boldsymbol{f}$$

2. Tissue samples:

$$\textbf{NO concentration } (\textbf{µmol/gprot}) = \frac{\Delta \boldsymbol{A_1} \times \boldsymbol{c}}{\Delta \boldsymbol{A_2}} \times \frac{\boldsymbol{f}}{\boldsymbol{C_{Protein}}}$$

where:

ΔA_1	$OD_{Sample} - OD_{Blank}$
ΔA_2	$OD_{Standard} - OD_{Blank}$
c	Sodium Nitrite concentration (40 µmol/l)
f	Dilution factor of sample
$C_{Protein}$	Sample protein concentration (gprot/l)