Nitric Oxide (NO) Assay Kit

Catalog No.: abx298829

Size: 100 Assays

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

Application: For quantitative detection of Nitric Oxide concentrations in serum, plasma, tissue homogenates, cell lysates, cell culture supernatants, urine, and other biological fluids.

Detection Range: 2 µmol/L – 200 µmol/L

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 Assay Kit is a quick, convenient, and sensitive method for measuring and calculating NO concentrations. The dye reagents react with NO to create an absorption maximum at 550 nm. The intensity of the color is proportional to the concentration of NO, which can then be calculated.

Kit components

1. 96 well microplate
2. Assay Buffer 1: 30 ml
3. Assay Buffer 2: 30 ml
4. Dye Reagent A: 1 vial
5. Dye Reagent A Diluent: 5 ml
6. Dye Reagent B: 1 vial
7. Standard: 1 vial
8. Plate sealer: 3

Materials Required But Not Provided

1. Microplate reader (550 nm)
2. Centrifuge and microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Timer
6. Ice
7. Sonicator
8. Mortar
9. Water bath

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Dye Reagent A Solution**
  Add 5 ml of Dye Reagent A Diluent into the Dye Reagent A vial and mix thoroughly to prepare the Dye Reagent A Solution. If any precipitates are observed, warm the vial using a water bath until the precipitates have dissolved.

- **Dye Reagent B Solution**
  Add 5 ml of distilled water into the Dye Reagent B vial and mix thoroughly to prepare the Dye Reagent B Solution. Ensure that the Dye Reagent has completely dissolved prior to use.

- **Standard Solution**
  Add 1 ml of distilled water into the Standard vial and mix thoroughly. Ensure that the Standard has completely dissolved. Take 2 µl of this solution and add 998 µl of distilled water to prepare the Standard Solution (concentration 200 µmol/L). Unused Standard Solution can be stored at 4°C.

2. Sample

- **Cell and Bacterial samples**
  Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge to precipitate the cells. Discard the supernatant and add 500 µl of distilled water for every 5,000,000 cells. Sonicate at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Centrifuge at 12,000 × g at 4°C for 20 minutes. Add 250 µl of Assay Buffer 1 and mix thoroughly. Add 250 µl of Assay Buffer 2 and mix thoroughly. Centrifuge at 10,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

- **Tissue samples**
  Homogenize 0.1 g of sample in 500 µl of distilled water. Add the homogenate to a centrifuge tube, add 250 µl of Assay Buffer 1 and mix thoroughly. Add 250 µl of Assay Buffer 2 and mix thoroughly. Centrifuge at 10,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

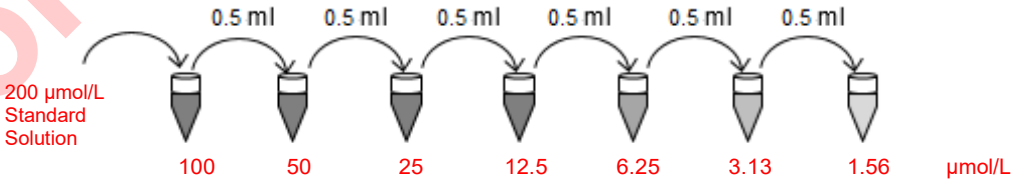
- **Liquid samples**
  Add 500 µl of sample to a centrifuge tube followed by 250 µl of Assay Buffer 1. Mix thoroughly. Add 250 µl of Assay Buffer 2, then mix thoroughly. Centrifuge at 10,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately. (Note: dilution factor is 2 if Assay Buffer 1 and 2 are added to the sample.)

B. Assay Procedure

Bring all reagents to room temperature prior to use.

If the expected concentration is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured concentration within the detection range of the kit.

1. Label 7 tubes with 100 µmol/L, 50 µmol/L, 25 µmol/L, 12.5 µmol/L, 6.25 µmol/L, 3.13 µmol/L, and 1.56 µmol/L. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 200 µmol/L Standard Solution to the 1st tube and mix thoroughly. Transfer 0.5 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on.



2. Set the sample, standard and blank wells on the 96 well microplate and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 50 µl of sample to the sample wells.
4. Add 50 µl of prepared standards to the standard wells.
5. Add 50 µl of distilled water to the blank wells.
6. Add 50 µl of Dye Reagent A Solution to all wells.
7. Tap the plate gently to mix. Allow to stand for 10 minutes.
8. Add 50 µl of Dye Reagent B Solution to all wells.
9. Tap the plate gently to mix. Allow to stand for 5 minutes. Read and record absorbance at 550 nm.

C. Calculations

Nitric Oxide concentration per mg of protein:

$$\textbf{Nitric Oxide } (\mu\text{mol/mg}) = \frac{C_{Standard} \times V_{Standard}}{V_{Sample} \times C_{Protein}} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} = \frac{0.2}{C_{Protein}} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}}$$

Nitric Oxide concentration per g of sample:

$$\textbf{Nitric Oxide } (\mu\text{mol/g}) = \frac{C_{Standard} \times V_{Standard} \times V_{Assay}}{V_{Sample} \times W} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} = \frac{0.2}{W} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}}$$

Nitric Oxide concentration per 10^4 cells or bacteria:

$$\textbf{Nitric Oxide } (\mu\text{mol}/10^4 \text{ cells}) = \frac{C_{Standard} \times V_{Standard} \times V_{Assay}}{V_{Sample} \times N} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} = \frac{0.2}{N} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}}$$

Nitric Oxide concentration per ml serum or plasma:

$$\textbf{Nitric Oxide } (\mu\text{mol/ml}) = \frac{C_{Standard} \times V_{Standard}}{V_{Sample}} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times n = 0.4 \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}}$$

where:

$C_{Protein}$	Concentration of protein (in mg/ml)
$C_{Standard}$	Concentration of highest standard (200 µmol/L= 0.2 µmol/ml)
W	Weight of the sample (in g)
N	Number of cells or bacteria (× 10^4)
V_{Assay}	Total volume of Assay Buffer (1 ml)
V_{Sample}	Volume of sample (0.05 ml)
$V_{Standard}$	Volume of standard (0.05 ml)
n	Dilution factor (2)