Nitrite Assay Kit

Catalog No.: abx298918

Size: 100 tests

Detection Range: 1.36 µmol/L – 500 µmol/L

Sensitivity: 1.36 µmol/L

Storage: Store the Standard at -20°C and the rest of the components in the dark at 4°C for up to 12 months.

Application: For detection and quantification of Nitrite concentration in serum, plasma, tissue, cell lysates, cell culture supernatants and saliva.

Introduction

Nitrites and Nitrates are ubiquitous ions that are produced in the nitrogen cycle, and are used widely in the chemical and pharmaceutical industries. Nitrites form a conjugate acid in water, and has redox activity. Nitrite reacts with the Detection Reagents in this assay to produce a light-red azo-compound.

Abbexa's Nitrite Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Nitrite concentration. The azo-compound product has an absorbance maxima at 550 nm. The intensity of the color is proportional to the Nitrite concentration, which can then be calculated.

Kit components

1. Assay Buffer: 2 × 50 ml
2. Alkali Reagent: 50 ml
3. Acid Reagent: 12 ml
4. Detection Reagent A: 1 vial
5. Detection Reagent B: 1 vial
6. Standard: 1 vial
7. Plate sealer: 2

Materials Required But Not Provided

1. Spectrophotometer (550 nm)
2. Double distilled water
3. Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. Vials/tubes
6. Sonicating water bath
7. Centrifuge
8. Vortex mixer
9. Incubator

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 4°C or at room temperature for up to 1 hr. 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.
- **Cell culture supernatant:** Centrifuge at 3100 × g for 10 minutes. Take the supernatant, keep on ice and assay immediately. If there is precipitation in the supernatant, centrifuge again.
- **Tissue Homogenates:** Weigh 0.02-1 g of tissue and wash with pre-chilled PBS. For each 1 g of tissue, add 9 ml of pre-chilled normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 1500 x g at 4°C for 10 min. Collect the supernatant and assay immediately. The protein concentration in the supernatant should be determined separately.
- **Cell lysates:** Collect cells into a centrifuge tube and wash with PBS. Centrifuge at 1000 × g for 10 min and discard the supernatant. Add 300-500 µl of normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) per 1 × 10^6 cells, then sonicate in an ice water bath. Centrifuge at 10,000 × g at 4 °C for 10 min. Take the supernatant into a new centrifuge tube (while kept on ice) and analyze immediately. The protein concentration in the supernatant should be determined separately.
- **Saliva:** Collect fresh saliva into a sterile container, then centrifuge at 10,000 × g at 4°C for 10 min. Take the supernatant, keep on ice and assay immediately.

Samples should not contain detergents such as SDS, Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT. Samples should not contain dyes such as phenol red.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment.

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

- **Detection Reagent A Solution:** Dissolve a vial of Detection Reagent A with 30 ml double distilled water at 65°C and mix fully. Unused Detection Reagent A Solution can be stored at 2-8°C for up to 3 months.
- **Detection Reagent B Solution:** Dissolve a vial of Detection Reagent B with 12 ml double distilled water and mix fully. Unused Detection Reagent A Solution can be stored at 2-8°C for up to 2 months. If the solution is a dark colour, it has degraded.
- **Detection Reagent Solution:** Mix Detection Reagent A Solution, Detection Reagent B Solution and Acid Reagent according to the ratio 2.5:1:1 (e.g. mix 2.5 ml of Detection Reagent A Solution, 1 ml of Detection Reagent B Solution and 1 ml of Acid Reagent to make 4.5 ml of Detection Reagent Solution). Prepare immediately before carrying out the assay at 2-8°C and mix fully. If the solution is a dark colour, it has degraded.
- **100 µmol/L standard solution**: Dissolve a vial of Standard with 2 ml double distilled water and mix fully to prepare a 2 mmol/L Standard. Dilute the 2 mmol/L Standard with double distilled water 20-fold and mix fully (e.g. add 19 ml double distilled water to 2 ml of 2 mmol/L Standard) to prepare the 100 µmol/L standard solution. Prepare immediately before carrying out the assay and mix fully.

B. Assay Procedure

1. Set blank, standard, sample tubes. It is recommended to measure in duplicate for each assay run. *Pipette samples up and down to mix before adding to wells. Avoid foaming or bubbles.*
2. **Serum and plasma samples**: add 0.3 ml of double distilled water to the blank tubes. Add 0.3 ml of 100 µmol/L standard solution to the standard tubes. Add 0.3 ml of sample to each sample tube.
3. **Tissue homogenates and cell lysates**: add 0.15 ml of double distilled water to the blank tubes. Add 0.15 ml of 100 µmol/L standard solution to the standard tubes. Add 0.15 ml of sample to each sample tube.
4. Add 0.8 ml of Assay Buffer to all tubes. Mix fully with a vortex.
5. Add 0.4 ml of Alkali Reagent to all tubes. Mix fully with a vortex.
6. Centrifuge at 2000 × g for 10 minutes. Take 0.8 ml of the supernatant to new tubes. If the supernatant contains precipitation, centrifuge again.
7. Add 0.4 ml of Detection Reagent Solution to all tubes. Mix fully, and stand at room temperature for 15 min.
8. Set the spectrophotometer to zero using double distilled water.
9. Measure the OD of each tube at 550 nm with a 0.5 cm optical path cuvette.

B. Calculation of Results

1. Serum and plasma samples:

$$NO_2^- \ (\mu mol/L) = \frac{(OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank})} \times C_{Standard} \times f$$

2. Tissues and cell lysate samples:

$$NO_2^- \ (\mu mol/g\ prot) = \frac{(OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank})} \times \frac{C_{Standard} \times f}{C_{Protein}}$$

where:

$OD_{Standard}$	OD value of standard
OD_{Sample}	OD value of sample
OD_{Blank}	OD value of blank
$C_{Standard}$	Concentration of the standard (100 μmol/L)
$C_{Protein}$	Concentration of protein in sample
f	The dilution factor of sample