Ammonia/Ammonium Assay Kit

Catalog No.: abx298905

Size: 100 Assays

Storage: Store all components at 4°C.

Application: For quantitative detection of Ammonia/Ammonium concentration in serum, plasma, tissue homogenates, cell lysates, cell culture supernatants, urine, saliva and other biological fluids.

Detection Range: 1 µmol/L – 1000 µmol/L

Introduction: Ammonia (NH_3), and its ionic form ammonium (NH_4^+), is an important source of nitrogen for living systems though is toxic at high concentrations. It is synthesized through amino acid metabolism. In the liver, ammonia is converted to urea through the urea cycle. Elevated concentrations of ammonia in the blood (hyperammonemia) is implicated in liver dysfunction (cirrhosis), while low levels of ammonia (hypoammonemia) is associated with defects in the urea cycle enzymes (for example, ornithine transcarbamylase).

Abbexa's Ammonia/Ammonium Microplate Assay Kit is designed to directly measure ammonia and ammonium in a variety of samples. In this assay, ammonia reacts with hypochlorous acid. The concentration of hypochlorous acid is directly proportional to the ammonia/ammonium concentration in the sample and can be calculated by measuring the absorbance at 620 nm.

Kit components

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

Materials Required But Not Provided

1. Microplate reader (620 nm) and incubator
2. Centrifuge and microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Timer
6. Ice
7. Sonicator
8. Mortar

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Dye Reagent 1 Solution**
  Add 7 ml of distilled water into the Dye Reagent 1 vial and mix thoroughly to prepare the Dye Reagent 1 Solution. Ensure that the Dye Reagent 1 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 5 µl of this solution and to 995 µl of distilled water to prepare the Standard Solution (concentration 1000 µmol/L).

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 1 ml of Assay Buffer 1 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 10,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube and add 0.5 ml of Assay Buffer 2. Mix thoroughly, then analyze immediately.

- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of Assay Buffer 1. Centrifuge at 10,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube and add 0.5 ml of Assay Buffer 2. Mix thoroughly, then analyze immediately.

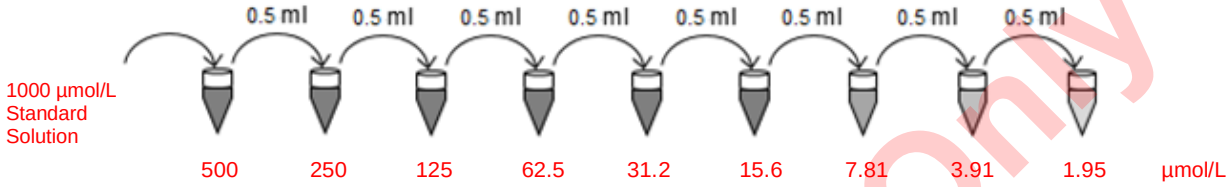
- **Serum, Plasma, Urine, and Other biological fluid samples**
  Liquid samples can be used directly.

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 9 tubes with 500 µmol/L, 250 µmol/L, 125 µmol/L, 62.5 µmol/L, 31.2 µmol/L, 15.6 µmol/L, 7.81 µmol/L, 3.91 µmol/L, and 1.95 µmol/L. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 1000 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 100 µl of sample to the sample wells.
4. Add 100 µl of prepared standards to the standard wells.
5. Add 100 µl of distilled water to the blank wells.
6. Add 70 µl of Dye Reagent 1 Solution to all wells.
7. Add 30 µl of Dye Reagent 2 to all wells.
8. Tap the plate gently to mix. Incubate at 37°C for 15 minutes.
9. Read and record absorbance at 620 nm.

C. Calculations

Ammonia/Ammonium concentration per mg of protein:

$$\mathbf{NH_3\ (\mu mol/mg)} = \frac{\mathbf{C_{Standard}} \times \mathbf{V_{Standard}}}{\mathbf{V_{Sample}} \times \mathbf{C_{Protein}}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}} = \frac{\mathbf{1000}}{\mathbf{C_{Protein}}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}}$$

Ammonia/Ammonium concentration per g of sample:

$$\mathbf{NH_3\ (\mu mol/g)} = \frac{\mathbf{C_{Standard}} \times \mathbf{V_{Standard}} \times \mathbf{V_{Assay}}}{\mathbf{V_{Sample}} \times \mathbf{W}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}} = \frac{\mathbf{1500}}{\mathbf{W}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}}$$

Ammonia/Ammonium concentration per L of sample:

$$\mathbf{NH_3\ (\mu mol/L)} = \frac{\mathbf{C_{Standard}} \times \mathbf{V_{Standard}}}{\mathbf{V_{Sample}}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}} = \mathbf{1000} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}}$$

where:

$\mathbf{C_{Protein}}$	Concentration of protein (in mg/ml)
$\mathbf{C_{Standard}}$	Concentration of highest standard (1000 µmol/L)
$\mathbf{W}$	Weight of the sample (in g)
$\mathbf{V_{Assay}}$	Volume of assay buffer (1.5 ml)
$\mathbf{V_{Sample}}$	Volume of sample (100 µl)
$\mathbf{V_{Standard}}$	Volume of standard (100 µl)