Product Manual

Ammonia Assay Kit (Fluorometric)

Catalog Number

MET-5181 **200 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Ammonia is a compound composed of nitrogen and hydrogen (formula NH_3). It is a common waste product in aquatic organisms, and serves as a nutritional staple of terrestrial organisms by functioning as a food/fertilizer precursor. Approximately 88% of ammonia is used around the world as fertilizer, anhydrously or as its salts or solutions. Ammonia is a building block for the synthesis of many pharmaceutical products as well as commercial cleaning products. Ammonia can be used to synthesize compounds such as ethanolamine, ethyl carbamate, ammonium perchlorate, ammonium nitrate, formamide, dinitrogen tetroxide, alprazolam, hexamethylenetetramine, and ammonium bicarbonate.

In certain organisms, ammonia is synthesized from nitrogen found in the air by nitrogenase enzymes using a process called nitrogen fixation. Ammonia is also a resulting product of amino acid deamination catalyzed by enzymes such as glutamate dehydrogenase 1. Ammonia is commonly excreted in aquatic animals, while in humans' ammonia is converted to the less toxic molecule urea. Urea is one of the main components of urine. Many birds, reptiles, and insects excrete nitrogenous waste as uric acid. Ammonia also functions in both normal and abnormal animal physiology: it is made by normal amino acid metabolism mechanisms and is toxic in high concentrations. The mammalian liver converts ammonia to urea through a series of enzymatic reactions called the urea cycle. In liver dysfunctions such as cirrhosis, elevated amounts of ammonia in the blood may result (hyperammonemia). At the same time, defects in the enzymes responsible for the urea cycle, such as ornithine transcarbamylase, lead to high ammonia levels. Hyperammonemia contributes to the symptoms of hepatic encephalopathy such as confusion and coma. In addition, high ammonia levels can cause neurologic diseases observed in people with urea cycle defects and organic acidurias.

Cell Biolabs' Ammonia Assay Kit utilizes a fluorescent probe of ammonia molecules. Ammonia reacts with the probe to produce a fluorescent product that can be measured with a standard fluorometric plate reader at an excitation wavelength of 360 nm and an emission of 415 nm. Each kit provides sufficient reagents to perform up to 200 assays, including blanks, ammonia standards and unknown samples.

Note: Each sample replicate requires 2 assays, one treated with the Fluorometric Reagent mixture (FR) and one with the Negative Control Reagent (NC). Ammonia is calculated from the difference in RFU readings from the 2 wells.

Assay Principle

Cell Biolabs' Ammonia Assay Kit measures ammonia levels within urine, cells, or tissue lysates. Samples are compared to a known concentration of ammonium chloride standard within a 96-well microtiter plate format. Samples and standards react with a fluorescent sensor to produce a fluorescent product. After 15-30 minutes, the plate is read with a standard 96-well fluorometric microplate reader at an excitation wavelength of 360 nm and an emission of 415 nm (Figure 1). Higher fluorescent values correlate with high ammonia concentrations. Sample ammonia concentrations are determined by comparison with the known ammonium chloride standards. The kit has a sensitivity of 6.25 µM ammonia.

Related Products

1. MET-5086: Ammonia Assay Kit (Colorimetric)
2. STA-382: Urea Assay Kit
3. STA-375: Uric Acid/Uricase Assay Kit
4. STA-378: Creatinine Assay Kit

Kit Components (shipped at room temperature)

1. Ammonium Chloride Standard (Part No. 50861B): One 50 µL tube of an 80 mM solution
2. Assay Reagent (Part No. 51811A): One 4 mL clear bottle
3. Fluorometric Reagent (Part No. 51801B): Two 20 mL amber bottles
4. Negative Control Reagent (Part No. 51813B): Two 20 mL amber bottles

Materials Not Supplied

1. Deionized water
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. 37ºC incubator
6. Fluorometric microplate reader capable of reading at 360 nm excitation and 415 nm emission.

Storage

Upon receipt store the kit at 4ºC.

Preparation of Samples

Samples should be stored at -80ºC prior to performing the assay. The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design. A set of serial dilutions is recommended for samples to achieve optimal assay results.

- Urine: Urine samples with visible particulates should be centrifuged or filtered prior to testing. A minimum 1:50 dilution of urine samples into deionized water is recommended to achieve optimal assay results. Diluted samples should be used within 2 hours of preparation.
- Tissue or Lysates: Homogenize 20 mg of tissue or 2 x 10^6 cells in 1 mL of deionized water. Centrifuge at 14000 x g for 10 min to remove insoluble material. Samples can be tested directly or diluted with deionized water.

Preparation of Ammonium Chloride Standard Curve

Use the provided stock Ammonium Chloride Standard 80 mM solution to prepare a fresh series of the remaining ammonium chloride standards according to Table 1 below.

Tubes	80 mM Ammonium Chloride Standard (µL)	Deionized Water (µL)	Resulting Ammonium Chloride Concentration (µM)
1	5	995	400
2	250 of Tube #1	250	200
3	250 of Tube #2	250	100
4	250 of Tube #3	250	50
5	250 of Tube #4	250	25
6	250 of Tube #5	250	12.5
7	250 of Tube #6	250	6.25
8	0	500	0

Table 1. Preparation of Ammonium Chloride Standards.
Note: Do not store diluted ammonium chloride standard solutions.

Assay Protocol

Each ammonium chloride standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

Note: Samples other than the standards require two paired wells, one to be treated with the Fluorometric Reagent mixture (FR), and one with the Negative Control Reagent (NC). This controlled pairing will allow for the determination of background levels within the sample.

1. Add 100 µL of the diluted ammonium chloride standards or samples to the 96-well microtiter plate wells. Remember to provide two sample paired wells if testing for background.
2. Add 20 µL of Assay Reagent A to each well using either a multichannel pipette. Mix thoroughly and carefully to avoid foaming in the well.
3. Add 200 µL of the Fluorometric Reagent (FR) to the standards and one half of the paired sample wells each using a multichannel pipette. Mix the solution thoroughly and carefully to avoid foaming in the well.
4. Add 200 µL of the Negative Control Reagent (NC) to the standards and one half of the paired sample wells each using a multichannel pipette. Mix the solution thoroughly and carefully to avoid foaming in the well.
5. Incubate 15-30 minutes at 37ºC.
6. Read the plate at excitation wavelength 360 nm and emission wavelength 415 nm.

Example of Results

The following figures demonstrate typical Ammonia Assay Kit (Fluorometric) results. One should use the data below for reference only. This data should not be used to interpret actual sample results.

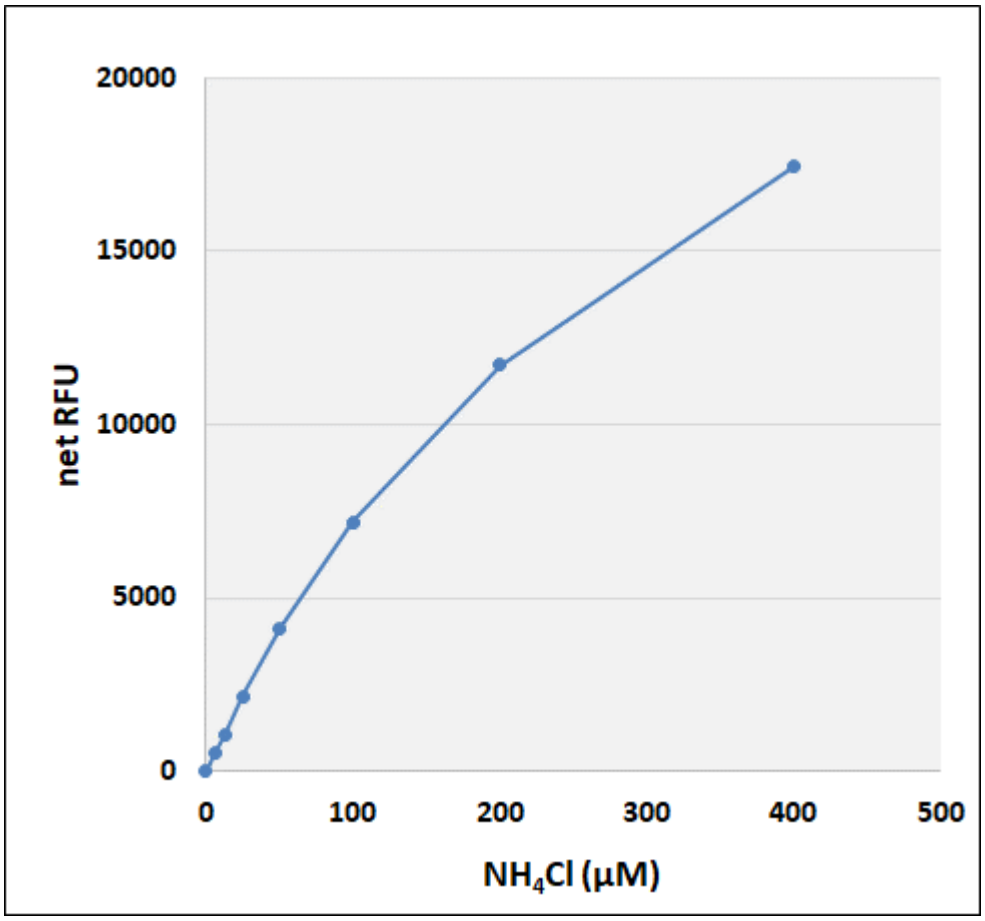

Figure 1: Ammonium Chloride Standard Curve.

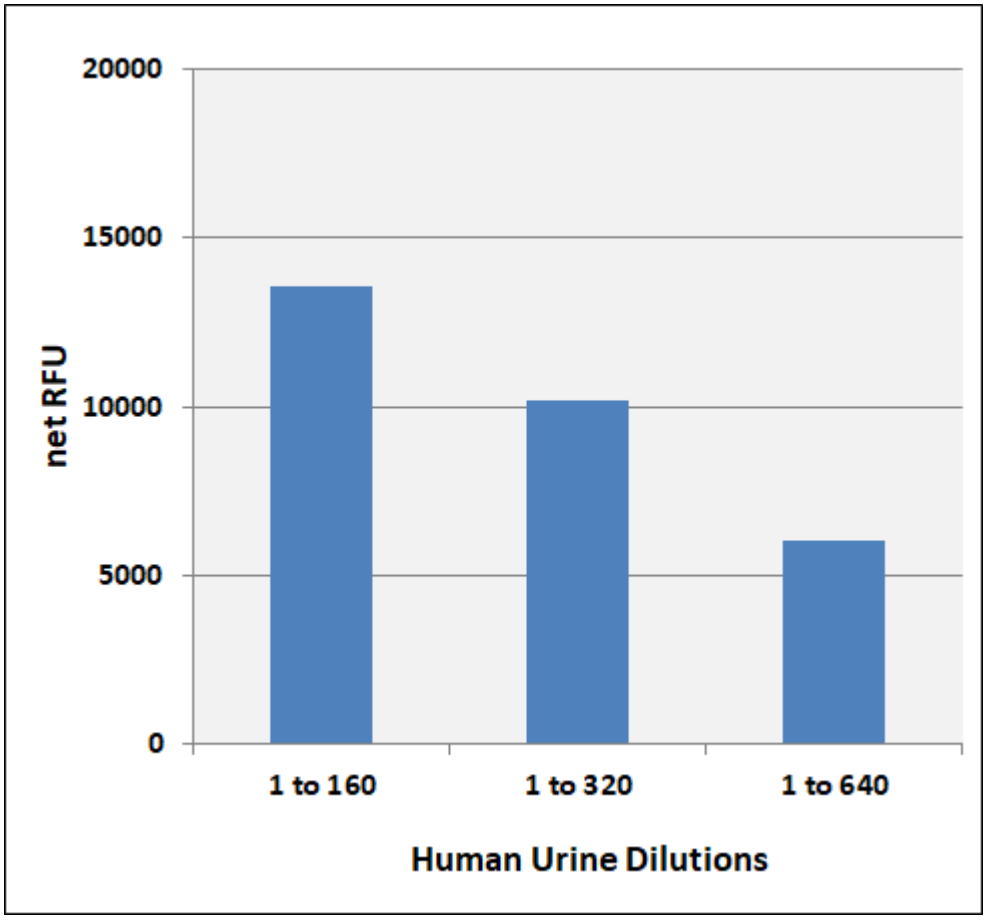

Figure 2: Urine Samples. Human urine samples were tested with the Ammonia Assay Kit.

Calculation of Results

1. Determine the average fluorescence values for each sample, control, and standard.

2. Subtract the average zero standard value from itself and all standard and sample values. This is the background correction.

3. Graph the standard curve (see Figure 1).

4. For samples with two paired wells (with Fluorometric Reagent or with Negative Control Reagent), subtract the sample well fluorescence values with Negative Control Reagent (F_{NC}) from the sample well fluorescence values with Fluorometric Reagent (F_{FR}) to obtain the fluorescence difference. The (F_{NC}) sample value represents the background concentration within the sample, while the (F_{FR}) sample value is the combined ammonia and background concentration within the sample. The fluorescence difference (ΔF) is due to the ammonia concentration:

$$(\Delta F) = (F_{FR}) - (F_{NC})$$

5. Compare the fluorescence values of each sample to the standard curve to determine and extrapolate the quantity of ammonia present in the sample. Only use values within the range of the standard curve.

References

1. Mus F, Crook MB, Garcia K, Garcia Costas A, Geddes BA, Kouri ED, Paramasivan P, Ryu MH, Oldroyd GE, Poole PS, Udvardi MK, Voigt CA, Ané JM, and Peters JW (2016) *Appl Environ Microbiol.* **82(13)**: 3698-3710.
2. Plaitakis A1, Kalef-Ezra E, Kotzamani D, Zaganas I, and Spanaki C. (2017). *Biology (Basel)* **6(1):** 1-26.
3. Weiner ID, Mitch WE, and Sands JM. (2015) *Clin J Am Soc Nephrol.* **10(8)**: 1444-1458.
4. Bigot A, Tchan MC, Thoreau B, Blasco H, and Maillot F. (2017) *J Inherit Metab Dis.* **40(6)**:757-769.
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Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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