Product Manual

Ammonia Assay Kit (Colorimetric)

Catalog Number

MET-5086 **100 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 is based on a modified version of the Berthelot reaction. Ammonia reacts with an alkaline reagent to produce a blue-green colored product that can be measured with a standard spectrophotometric plate reader at an optical density between 630-670 nm. Each kit provides sufficient reagents to perform up to 100 assays, including blanks, ammonia standards and unknown samples.

Assay Principle

Cell Biolabs' Ammonia Assay Kit measures ammonia levels within urine or other biological samples. Samples are compared to a known concentration of ammonium chloride standard within a 96-well microtiter plate format. Samples and standards react with a chromogen in alkali solution to produce a blue-green colored product. After 30 minutes, the plate is read with a standard 96-well spectrophotometric microplate reader at an optical density between 630 nm and 670 nm (Figure 2). Higher OD values correlate with high ammonia concentrations. Sample ammonia concentrations are determined by comparison with the known ammonium chloride standards. The standard curve is linear up to 800 µM ammonia and the kit has a sensitivity of 12.5 µM ammonia.

Related Products

1. STA-382: Urea Assay Kit
2. STA-375: Uric Acid/Uricase Assay Kit
3. STA-378: Creatinine Assay Kit

Kit Components

1. Ammonium Chloride Standard (Part No. 50861B): One 50 µL tube of an 80 mM solution
2. Assay Reagent A (Part No. 50862B): One 8 mL amber bottle
3. Assay Reagent B (Part No. 50863B): One 4 mL amber bottle

Materials Not Supplied

1. Standard 96-well microtiter plates for use in microplate reader
2. Deionized water

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 and minimize possible interfering chromogens.

- 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 $2x10^6$ 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.

Notes:

- *All samples must be free of exogenous ammonia containing salts or buffer systems.*
- *Hemoglobin (>200 mg/dL), Bilirubin (>20 mg/dL), and Triglycerides (>800 mg/dL) may interfere with the assay. Use controls accordingly.*

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.

Tubes	80 mM Ammonium Chloride Standard (µL)	Deionized Water (µL)	Resulting Ammonium Chloride Concentration (µM)
1	5	495	800
2	250 of Tube #1	250	400
3	250 of Tube #2	250	200
4	250 of Tube #3	250	100
5	250 of Tube #4	250	50
6	250 of Tube #5	250	25
7	250 of Tube #6	250	12.5
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.

1. Add 100 µL of the diluted ammonium chloride standards or samples to the 96-well microtiter plate wells.
2. Add 80 µL of Assay Reagent A to each well using either a multichannel pipette or a plate reader liquid handling system. Mix thoroughly and carefully to avoid foaming in the well.
3. Add 40 µL of Assay Reagent B to each well using either a multichannel pipette or a plate reader liquid handling system. Mix the solution thoroughly and carefully to avoid foaming in the well.
4. Incubate 30 minutes at 37ºC.
5. Read the plate at 630-670 nm.

Example of Results

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

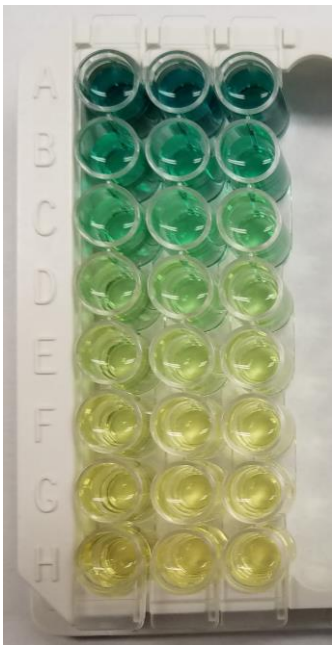

Figure 1: Color Visualization of Ammonia Assay Standard Curve.

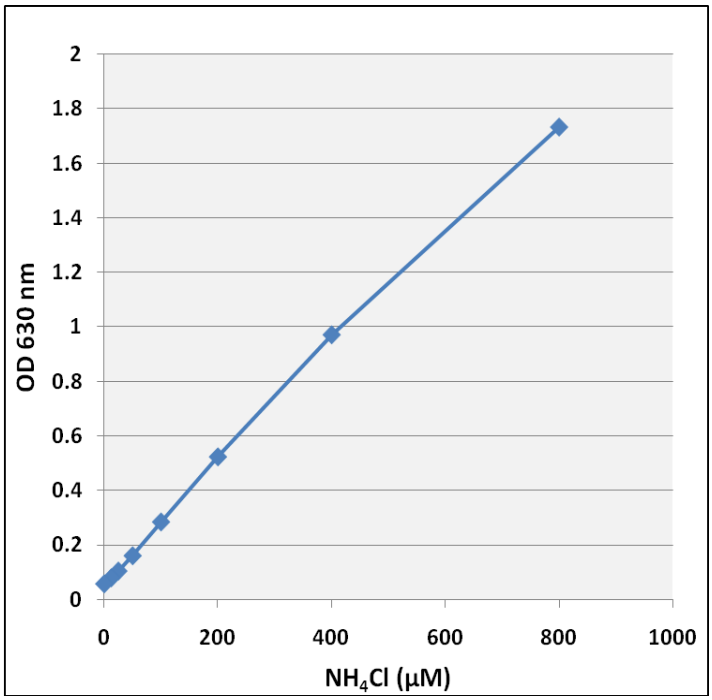

Figure 2: Ammonium Chloride Standard Curve.

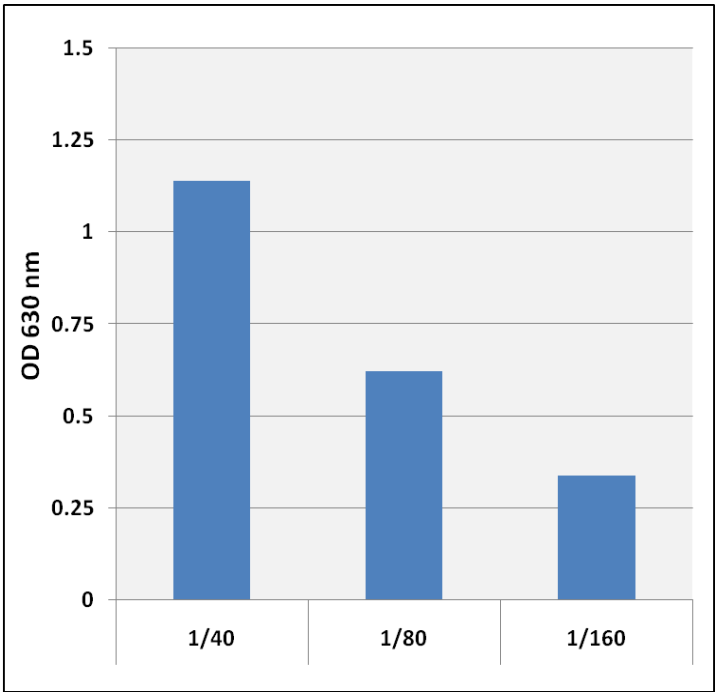

Figure 3: Urine Samples. Human urine samples were tested with the Ammonia Assay Kit.

References

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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.
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Recent Product Citations

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2. Suzuki, S. et al. (2021). Lipase and protease activities in Koji cheeses surface-ripened with Aspergillus strains. *J. Food Sci. Technol*. **27**(3):543-549. doi: 10.3136/fstr.27.543.

Warranty

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