Ammonia / Ammonium Assay Kit (Colorimetric)

Ammonia / Ammonium Assay Kit (Colorimetric) is a detection kit for the quantification of Ammonia / Ammonium in serum, plasma, urine, saliva and cell culture supernatants.

Catalog number: ARG82135

Package: 100 tests

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

The ammonium ion is generated when ammonia, a weak base, reacts with Brønsted acids (proton donors):

$H^+ + NH_3 \rightarrow NH_4^+$

The ammonium ion is mildly acidic, reacting with Brønsted bases to return to the uncharged ammonia molecule:

$NH_4^+ + B^- \rightarrow HB + NH_3$

Thus, treatment of concentrated solutions of ammonium salts with strong base gives ammonia. When ammonia is dissolved in water, a tiny amount of it converts to ammonium ions:

$H_2O + NH_3 \rightleftharpoons OH^- + NH_4^+$

Ammonium ions are a waste product of the metabolism of animals. In fish and aquatic invertebrates, it is excreted directly into the water. In mammals, sharks, and amphibians, it is converted in the urea cycle to urea, because urea is less toxic and can be stored more efficiently. In birds, reptiles, and terrestrial snails, metabolic ammonium is converted into uric acid, which is solid and can be excreted with minimal water loss.

Ammonium is an important source of nitrogen for many plant species, especially those growing on hypoxic soils. However, it is also toxic to most crop species and is rarely applied as a sole nitrogen source. [Provide by Wikipedia: Ammonium]

PRINCIPLE OF THE ASSAY

This Ammonia / Ammonium Assay Kit (Colorimetric) is a simple colorimetric assay that measures the amount of ammonia and ammonium present in serum, plasma, urine, saliva and cell culture supernatants. In this assay, NADH is converted to NAD^+ in the presence of NH_3, ketoglutarate and glutamate dehydrogenase. The decrease in optical density at O.D. 340 nm or fluorescence intensity at λex/em = 360/450 nm is directly proportionate to the NH_3 concentration in the sample.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped on ice. Store all components at -20°C. Shelf life of six months after receipt, 3 weeks after reconstitution.

Component	Quantity	Storage information
Assay Buffer	20 mL	-20°C
Ketoglutarate	120 µL	-20°C
NADH Reagent (lyophilized)	1 vial	-20°C
Enzyme	120 µL	-20°C
Standard	400 µL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 340 nm
- Fluorescence microplate reader capable of reading excitation at 350-360 nm and emission at 450 nm.
- Centrifuge
- Clear and black flat-bottom 96 well microplate
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C. Collect the serum and assay directly.

Plasma: Collect blood with EDTA, heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C. Collect the plasma layer and assay directly.

Cell culture media: dilute 5-10 fold in distilled water prior to assay.

Solid sample: sample extracted by homogenization in distilled water and filtered, centrifuged or, if necessary, deproteinized to remove any undissolved material. Samples should be clear and colorless with pH adjusted to 7 - 8.

REAGENT PREPARATION

- **NADH Reagent:** Reconstitute the NADH Reagent tube with 1000 µL of distilled water (final conc. 10 mM). Unused reconstituted NADH reagent is stable for three weeks when stored at -20°C.
- **Working Reagent:** for each 96-well reaction, mixing 180 µL of Assay Buffer, 1 µL of Enzyme, 8 µL of reconstituted NADH Reagent and 1 µL of Ketoglutarate.
- **Blank Control Reagent:** for each 96-well reaction, mixing 180 µL of Assay Buffer, 8 µL of reconstituted NADH Reagent and 1 µL of Ketoglutarate. (No enzyme)
- **Standard:** Prepare a 1000 µM NH_3 Standard Premix by mixing 15 µL of the 20 mM Standard and 285 µL of distilled water. Dilute Standard as follows.

Standard tube	Final NH_3 conc. (µM)	Distilled water (µL)	20 mM standard
S1	1000	0	100
S2	600	40	60
S3	300	70	30
S4	0	100	0

ASSAY PROCEDURE

Prior to the assay, equilibrate all components to room temperature. Briefly centrifuge all tubes before opening.

Colorimetric procedure (clear flat-bottom 96-well microplate):

	Sample	Standard	Sample blank
Diluted Standard		20 µL	
Samples	20 µL		20 µL
Working Reagent	180 µL	180 µL	
Blank Control Reagent			180 µL
Tap microplate to mix. Incubate for 30 minutes at room temperature. Read the absorbance at O.D. 340 nm.			

Fluorimetric procedure (black flat-bottom 96-well microplate):

	Sample	Standard	Sample blank
Diluted Standard		20 µL	
Samples	20 µL		20 µL
Working Reagent	180 µL	180 µL	
Blank Control Reagent			180 µL
Tap microplate to mix. Incubate for 30 minutes at room temperature. Read excitation at 350-360 nm and emission at 450 nm with a fluorescence reader.			

CALCULATION OF RESULTS

1. Subtract the standard values from the blank value (#4) and plot the ΔOD or ΔF against standard concentrations. Determine the slope and calculate the NH_3 concentration of Sample,

   Ammonia (μM) = [(R_{Blank} – R_{Sample}) / Slope (μM^{-1})] x n

   Note:

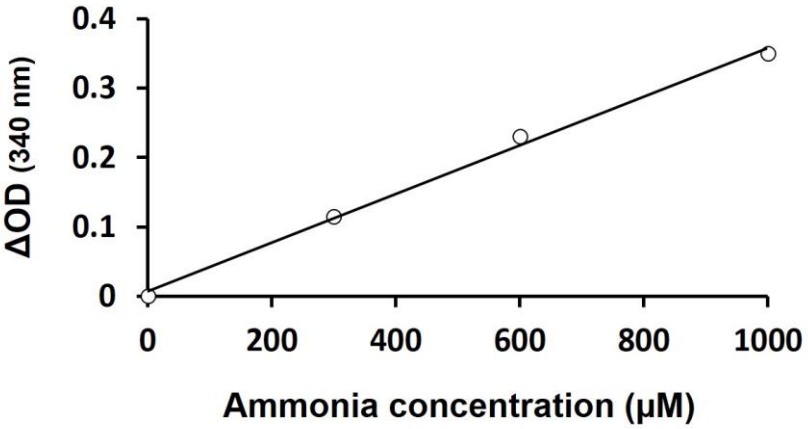
   R_{Sample} and R_{Blank}: Optical density of fluorescence intensity reading of the Sample and Sample Blank.

   n: Dilute factor.

2. If the calculated NH_3 concentration is higher than 1000 μM, dilute sample in dH2O and repeat assay. Multiply result by the dilution factor n.
3. Conversions: 1000 μM NH_3 equals 1.7 mg/dL or 17 ppm.

EXAMPLE OF TYPICAL STANDARD CURVE

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



QUALITY ASSURANCE

Sensitivity

24 μM