Ammonia Colorimetric Assay Kit II

(A Modified Berthelot Assay)

(Catalog #K470-100; 100 assays; Store kit at +4°C)

I. Introduction:

Ammonia is an important source of nitrogen for living systems. Nitrogen is required for the synthesis of amino acids, which are the building blocks of protein. Ammonia is a metabolic product which is created through amino acid deamination. It plays an important role in both normal and abnormal animal physiology such as acid/base balance. BioVision provides a rapid, simple, sensitive, and reliable assay suitable for research and high throughput assay of Ammonia or Ammonium. In this non-enzymatic assay, ammonia or ammonium is used to form indophenol, a highly colored product easily quantifiable by colorimetry ($OD_{670\ nm}$) using a plate reader. The kit can detect less than 1 nmol (~10 μM) ammonia or ammonium using either a 96 well or 384 well microwell plate.

II. Kit Contents:

Components	100 assays	Color Code	Part Number
Ammonia Reagent 1	8 ml	Amber	K470-100-1
Ammonia Reagent 2	4 ml	Clear	K470-100-2
Ammonium Chloride Standard (100 mM)	0.1 ml	Yellow	K470-100-3

III. Storage and Handling:

Store the kit at +4°C. Read the entire protocol before performing the assay. **All solutions should be kept tightly capped when not in use to prevent absorption of ammonia from the air.**

IV. Reagent preparation:

The two Ammonia reagents provided are ready to use as supplied.

V. Ammonia Assay Protocol:

1. **Standard Curve Preparation:**

   Dilute the Ammonium Chloride Standard solution to 1 mM by adding 10 µl of the 100 mM Ammonium Chloride Standard to 990 µl of dH_2O, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells. Adjust volume to 100 µl/well with water to generate 0, 2, 4, 6, 8, 10 nmol/well of the Ammonium Chloride Standard.

2. **Sample Preparations:**
   Liquid samples such as sea water, soil extracts, etc. can be tested directly. Add 2-100 μl sample to a 96 well plate; bring the volume to 100 µl/well with ammonia-free water. For unknown samples, we suggest testing several different doses of samples to make sure the readings are within the standard curve range.

3. **Reaction:** Add 80 µl of Reagent 1 to each standard and sample well. Add 40 µl of Reagent 2 to each well. Incubate at 37°C for 30 minutes.

4. **Read:** Measure OD at 670 nm in a microplate reader.

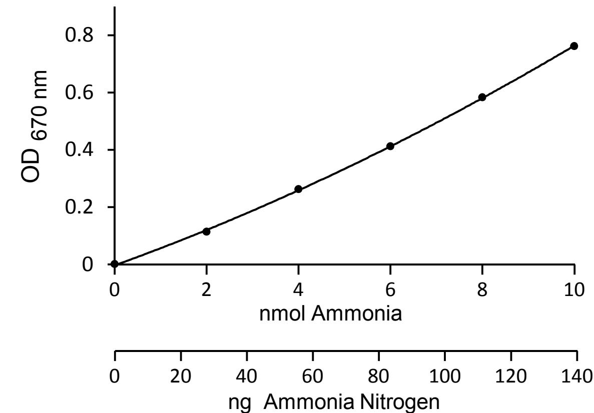
5. **Calculation:** Correct background by subtracting the value derived from the 0 Ammonium Chloride Standard from all readings (The background reading can be significant and must be subtracted). Plot the Ammonium Chloride Standard curve. Ammonium Chloride sample concentrations can then be calculated:

$$C = S_a/S_v \quad \text{nmol/µl or mM},$$

Where: S_a is the sample amount (in nmol) from standard curve

S_v is the sample volume (µl) added into the wells

NH_4^+ Molecular Weight is 18.04 g/mol



Standard curve generated in standard flat-bottom 96 well plates

Notes:

1) Amines and amides may interfere with the Berthelot assay and should be tested for interference if significant concentrations are expected in samples. The reagents used for the BioVision Ammonia assay were selected to minimize interference from non-ammonia sources (Refer to Figure 5 of reference 1 for more details).
2) Biological samples should be deproteinized prior to testing. In general we recommend using a spin filter (i.e. BioVision Cat.#1997-25) rather than acid precipitation to remove proteins since acid can deamidate proteins leading to higher ammonia background levels.

RELATED PRODUCTS:

NAD(P)/NAD(P)H Quantification Kit	ADP/ATP Ratio Assay Kit
Ascorbic Acid Quantification Kit	Glutathione Detection Kits
Glucose Assay Kit	Fatty Acid Assay Kit
Uric Acid Assay Kit	Alanine Assay Kit
Pyruvate Assay Kit	Lactate Assay Kit/ II
Triglyceride Assay Kit	Phosphate Assay Kit
Choline/Acetylcholine Quantification Kit	Hemin Assay Kit
Antioxidant Capacity (TAC) Assay Kit	Glycogen Assay Kit
L-amino Acid Assay Kit	Nitric Oxide Assay Kits
Ethanol Assay Kit	Urea Assay Kit
Sarcosine Assay Kit	Creatinine Assay Kit
Creatine Assay Kit	Lactate Assay Kits
SOD Assay Kit	Hydrogen Peroxide Assay Kit
G6PD Assay Kit	Glutathione Dehydrogenase Assay Kit
Glutathione Reductase Assay Kit	Nitric Oxide Assay Kits

References:

1) E.D. Rhine, G.K. Pratt, R.L. Mulvaney and E.J. Pratt (1998) Soil Sci. Soc. AM. J. 62: 473-480

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold reagents • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Reagents must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in datasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		