Ammonia Colorimetric Assay Kit II (#BN00704)

(A Modified Berthelot Assay)

(Catalog #BN00704; 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. Assay Genie 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	BN00704-1
Ammonia Reagent 2	4 ml	Clear	BN00704-2
Ammonium Chloride Standard (100 mM)	0.1 ml	Yellow	BN00704-3

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.

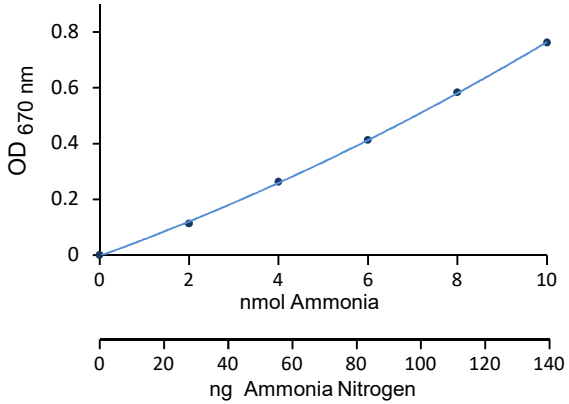
5. **Calculation:** Correct background by subtracting the value derived from the 0 Ammonium Chloride Standard from all readings (The background reading can be significant and must be subtracted). Plot the Ammonium Chloride Standard curve. Ammonium Chloride sample concentrations can then be calculated:

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

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 Assay Genie 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 rather than acid precipitation to remove proteins since acid can deamidate proteins leading to higher ammonia background levels.

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