MA-0140

Ammonia Assay II (Modified Berthelot Assay)

(100 wells, Colorimetric, OD 670 nm, Store at 4°C)

Introduction:

Ammonia is an important both as a source of and a means of eliminating nitrogen in living systems. Required for the synthesis of amino acids, as well as nucleotides and some lipids, ammonia is typically formed through amino acid deamination. In the liver, ammonia is converted to the much less toxic urea and in birds and reptiles, into uric acid. While not a buffer at physiological pH, it plays an important role in acid/base balance. AkrivisBio's Ammonia Assay II is a simple, sensitive, reliable assay with a sensitivity below 1 nmole per 100 µl well. In the assay, ammonia is converted to indophenol, with strong absorbance at 670 nm.

Assay Components:

Phenylphenol Reagent	8 ml	Amber	MA-0140A
Hypochlorite Reagent	4 ml	Clear	MA-0140B
Ammonium Standard	500 µl	Yellow	MA-0140C

Storage and Handling:

Store the kit at 4°C. Keep all containers tightly capped to prevent absorption of ammonia from the air.
All components are ready to use as supplied. Ammonium Standard is 0.4 mM. Store at room temperature.

Assay Protocol:

1. **Standard Curve:**
Transfer 0 – 5 – 10 – 15 – 20 - 25 µl of the Ammonium Standard to a series of wells in a 96-well plate. Adjust all well volumes to 100 µl with water giving 0, 2, 4, 6, 8, 10 nmol/well of Ammonium Standard.

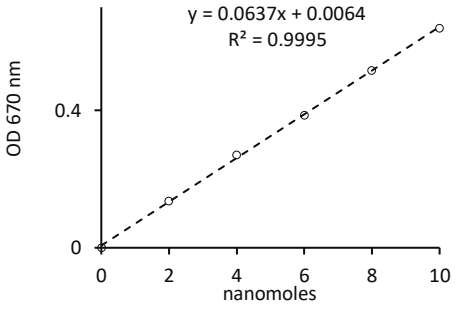
2. **Sample Preparation:**
Add liquid samples (5-100 µl) directly to test well, then bring all well volumes to 100 µl with high purity water. Samples which are not liquid should be diluted with high purity water and centrifuged or filtered to remove particulates and a sample of the liquid phase used.
Notes. Deproteinize biological samples before testing. Filtration is preferred over acid precipitation since the latter can cause deamidation.

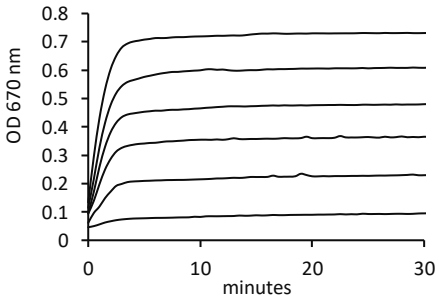
3. **Reaction:** Add 80 µl of Phenylphenol Reagent to all wells. Add 40 µl of Hypochlorite Reagent to each well. Incubate at 37°C for 20 - 30 minutes.

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

5. **Typical Results:**

nmole Standard	Standard Raw Values OD	Background Corrected Values OD
0	0.0899	0
2	0.2257	0.1358
4	0.3602	0.2703
6	0.4764	0.3865
8	0.6058	0.5159
10	0.7300	0.6401





6. **Calculation:** Subtract the zero standard from all other standards. Plot the standard curve. Determine the slope of the background corrected standard curve. The slot defines the sensitivity of the assay (absorbance per nmole). Subtract the value of the 0 standard from all test samples. Determine the ammonia content of the test sample wells by dividing the background-corrected absorbance by the slope of the standard curve = nmoles per well. To convert that value to ammonia per sample:

A. Divide the nmoles per well by the volume in µl of sample added to the test well = nmoles ammonia/µl of sample
B. Multiply the nmoles ammonia/µl of sample by the total volume of the sample or the volume of liquid obtained after centrifugation or filtration = total nmoles of ammonia per sample
C. Divide the total nmoles of ammonia per sample by the amount of tissue, # of cells, mg of other solid sample, etc.= nmoles of ammonia per amount of tissue (or # of cells or mg of sample, etc.)

References:

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

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