Amplite™ Colorimetric Ammonia/Ammonium Quantitation Kit
Blue Color

Ordering Information:	*Storage Conditions:*	*Instrument Platform:*
Product Number: 10059 (200 assays)	Keep in -20°C and avoid light	Absorbance microplate readers

Introduction

Ammonia (NH_3)/ammonium salt is an important source of nitrogen. In human, ammonia is produced through amino acid deamination and converted to urea through the urea cycle, which is then eliminated in urine. Ammonia levels in the blood rise when the liver is not able to convert ammonia to urea. Hyperammonemia, the elevated levels of ammonia in the blood, has been found in liver dysfunction (cirrhosis), while hypoammonemia has been associated with defects in the urea cycle enzymes (e.g. ornithine transcarbamylase). An ammonia test is usually important in clinical diagnostics to check how well the liver is working or the success of treatment for severe liver disease.

AAT Bioqest's Amplite™ Colorimetric Ammonia/Ammonium Assay Kit provides a rapid, simple and sensitive colorimetric method for the quantitation of ammonia/ammonium concentration in biological samples such as serum, plasma and urine. The assay is based on a chromogenic reaction to produce a blue product upon the reaction of ammonia with our sensor. The intensity of color produced is proportional to the concentration of ammonia in the sample, which can be measured at absorbance 660-670 nm. This kit provides a simple assay to detect as little as 4 µM ammonia in a 150 µL assay volume.

Kit Components

Components	Amount
Component A: Assay Buffer I	1 bottle (10 mL)
Component B: Assay Buffer II	1 bottle (10 mL)
Component C: Ammonium Chloride Standard (1.0 M)	1 vial (0.2mL)

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare ammonium standards or test samples (50 µL) → Add Assay Reaction Mixture I (50 µL) → Incubate at RT or 37 °C for 5 min → Add Assay Buffer II → Incubate at RT for 30-60 min → Read Absorbance at 665 nm

Note: Thaw all the kit components to room temperature before starting the experiment.

1. Prepare serial dilutions of Ammonium Chloride (0 to 1mM) solutions:

1.1 Add 1µL of 1.0 M Ammonium Chloride Standard (Component C) to 999 µL DPBS to generate 1.0 mM standard ammonium chloride solution.

1.2 Take 300 µL of 1.0 mM standard to perform 1:3 serial dilutions to get 300, 100, 30, 10, 3, 1, and 0 µM standard ammonium chloride solutions.

1.3 Add Ammonium Chloride Standards and ammonia containing test samples into a 96-well clear bottom microplate as described in Tables 1 and 2.

Table 1. Layout of standards and test samples in a clear bottom 96-well microplate:

BL	BL	TS	TS	….	….						
AS 1	AS 1	….	….	….	….						
AS 2	AS 2										
AS 3	AS 3										
AS 4	AS 4										
AS 5	AS 5										
AS 6	AS 6										
AS 7	AS 7										

Note: AS= Ammonium Chloride Standards, BL=Blank Control, TS=Test Samples.

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Table 2. Reagent composition for each well:

Ammonium Chloride Standard	Blank Control	Test Sample
Serial dilutions*: 50 µL	DPBS: 50 µL	50 µL

**Note: Add the serially diluted ammonium chloride standards from 1 to 1000 µM into wells from AS1 to AS7 in duplicate.*

2. Run Ammonium Chloride Assay:

2.1 Add 50 µL of Assay Buffer I (Component A) to each well of the Ammonium Chloride Standard, blank control, and test samples (see Step 1.3) so that the total assay volume is 100 µL/well.
Note: For a 384-well plate, add 25 µL sample, 25 µL of Assay Buffer I per well.

2.2 Incubate the reaction for 5 minutes at room temperature.

2.3 Add 50 µL of Assay Buffer II (Component B) to each well so that the total assay volume is 150 µL/well.
Note: For a 384-well plate, add 25 µL Assay Buffer II (Component B) to each well.

2.4 Incubate at room temperature for 30-60 minutes, and monitor the absorbance increase at 660-670 nm using an absorbance microplate reader.
Note: The color turns to yellow after Assay Buffer II (Component B) is added, and the wells with Ammonium Chloride Standard or samples will show bluish green color after incubation. The intensity of the color will reach the maximum in 30-60minutes.

Data Analysis

The absorbance in blank wells (with DPBS only) is used as a control, and is subtracted from the values for those wells with Ammonium Chloride Standards. The typical data are shown in Figure 1 (ammonium chloride standard curve).
Note: The absorbance background is subtracted from the absorbance intensity value of the wells for each data point.

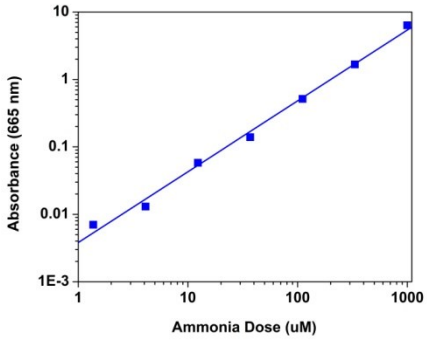

Figure 1. Ammonium chloride dose response in a 96-well clear bottom plate using a Spectrum Max microplate reader (Molecular Devices) measured with Amplite™ Colorimetric Ammonia/Ammonium Quantitation Kit. As low as 4 µM ammonia can be detected (n=3) in 45 minutes incubation after Assay Buffer II is added.

References

1. Goldbecker, Annemarie, et al. "Blood–brain barrier permeability for ammonia in patients with different grades of liver fibrosis is not different from healthy controls." *Journal of Cerebral Blood Flow & Metabolism* 30.7 (2010): 1384-1393.
2. Gorostiaga, Esteban M., et al. "Vertical jump performance and blood ammonia and lactate levels during typical training sessions in elite 400-m runners." *The Journal of Strength & Conditioning Research* 24.4 (2010): 1138.
3. Shinozaki, Koichiro, et al. "Blood ammonia and lactate levels on hospital arrival as a predictive biomarker in patients with out-of-hospital cardiac arrest." *Resuscitation* 82.4 (2011): 404-409.
4. Shawcross, Debbie L., et al. "Ammonia and the neutrophil in the pathogenesis of hepatic encephalopathy in cirrhosis." *Hepatology* 51.3 (2010): 1062-1069.