Amplite™ Colorimetric Ammonia Quantitation Kit *Blue Color*

Catalog number: 10059
Unit size: 200 Tests

Component	Storage	Amount
Component A: Assay Buffer I	Freeze (<-15 °C), Minimize light exposure	1 bottle (10 mL)
Component B: Assay Buffer II	Freeze (<-15 °C), Minimize light exposure	1 bottle (10 mL)
Component C: Ammonium Chloride Standard (1.0 M)	Freeze (<-15 °C)	1 vial (0.2mL)

OVERVIEW

Ammonia is an important source of nitrogen for living systems. It is synthesized through amino acid metabolism and is toxic when present at high concentrations. It is produced in liver and converted to urea through the urea cycle. Elevated levels of ammonia in the blood (hyperammonemia) have been found in liver dysfunction (cirrhosis), while hypoammonemia has been associated with defects in the urea cycle enzymes (e.g. ornithine transcarbamylase). The determination of ammonia is very useful test in clinical laboratory to monitor health status. Our Amplite™ Colorimetric Ammonia Assay Kit provides a simple and sensitive colorimetric method for the quantitation of ammonia concentration in foods and biological samples such as serum, plasma and urine, etc. The assay is based on an enzyme-coupled reaction of ammonia in the assay buffer, and finally produces a blue colored product. The intensity of color produced is proportional to the concentration of ammonia in the sample, which can be measured colorimetrically at 660-670 nm. This Amplite™ Colorimetric Ammonia Assay Kit provides a simple assay to detect ammonia. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step.

AT A GLANCE

Protocol summary

1. Prepare Ammonium Chloride standards or test samples (50 µL)
2. Add Assay Buffer I (50 µL)
3. Incubate at RT or 37°C for 5 min
4. Add Assay Buffer II (50 µL)
5. Incubate at RT for 30 - 60 min
6. Monitor Absorbance increase at 660 - 670 nm

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

KEY PARAMETERS

Instrument:	Absorbance microplate reader
Absorbance:	660 - 670 nm
Recommended plate:	Clear bottom

PREPARATION OF STANDARD SOLUTION

Ammonium Chloride standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/10059

Add 1 µL of 1.0 M Ammonium Chloride Standard (Component C) into 999 µL of DPBS to generate 1000 µM Ammonium Chloride standard solution (AS7). Take 1000 µM Ammonium Chloride standard solution (AS7) and perform 1:3 serial dilutions to get serially diluted Ammonium Chloride standards (AS6 - AS1) with DPBS.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of Ammonium Chloride standards and test samples in a clear bottom 96-well microplate. AS= Ammonium Chloride Standards (AS1 - AS7, 1 to 1000 µM), BL=Blank Control, TS=Test Samples.

BL	BL	TS	TS
AS1	AS1	...	...
AS2	AS2	...	...
AS3	AS3		
AS4	AS4		
AS5	AS5		
AS6	AS6		
AS7	AS7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
AS1 - AS7	50 µL	Serial Dilutions (1 to 1000 µM)
BL	50 µL	DPBS
TS	50 µL	test sample

1. Prepare Ammonium Chloride standards (AS), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

2. Add 50 µL of Assay Buffer I (Component A) to each well of Ammonium Chloride standard, blank control, and test samples to make the total assay volume of 100 µL/well. For a 384-well plate, add 25 µL of Assay Buffer I into each well instead, for a total volume of 50 µL/well.

3. Incubate the reaction at room temperature or 37°C for 5 minutes.

4. Add 50 µL of Assay Buffer II (Component B) to each well to make the total assay volume of 150 µL/well. For a 384-well plate, add 25 µL of Assay Buffer II into each well instead, for a total volume of 75 µL/well.

5. Incubate the reaction at room temperature for 30 - 60 minutes.

6. Monitor the absorbance increase with an absorbance microplate reader at 660 - 670 nm.

   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 - 60 minutes.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (Absorbance) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a

Tel: 408-733-1055 | Fax: 408-733-1304 | Email: support@aatbio.com

standard curve and equation. This equation can be used to calculate Ammonia samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

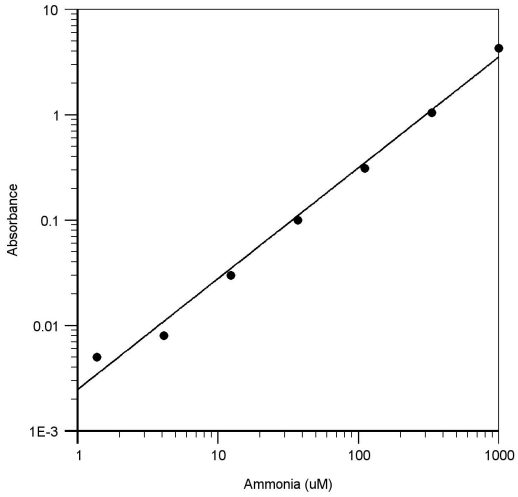

Figure 1. Ammonia dose response on a 96-well white clear bottom plate using a Spectrum Max microplate reader (Molecular Devices) measured with Amplite™ Colorimetric Ammonia/Ammonium Quantitation Kit.

DISCLAIMER