ABS_Bio™ Ammonia Detection Kit

(Cat# K103-200; 200 assays; store kit at 4°C)

Introduction

Ammonia is a nitrogen waste compound that is normally excreted in the urine. An elevated blood ammonia level occurs when the kidneys or liver are not working properly, allowing waste to remain in the bloodstream.

The ABS_Bio™ Ammonia Detection Kit provides a simple, sensitive, one-step colorimetric and fluorimetric assay to detect ammonia in plasma, serum, urine, saliva, and environment samples. In this assay, ammonia was reacted with phthalaldehyde and mercaptoethanol to form a colored products which can be measured by spectrophotometry or fluorometry. The intensity of color, measured at 420 nm or fluorescence intensity measured at Ex/Em=410/470nm, is directly proportional to the ammonia in the sample. The kit is supplied with sufficient reagents for 200 tests in 96-well plate assay, linear detection range of 0.018-5 mM (0.27-500 µM fluorimeter). It could easily be modified for use in 384-well assay and high-throughput assay.

Kit Components (200 tests)

Assay Buffer: 20 mL NH_4Cl Standard (100 mM): 0.5 mL Reagent A: 0.9 mL Reagent B: 0.9 mL

Storage and Handling: Store kit at 4°C. Shelf Life: 6 months after receipt. Warm up Reagents to room temperature before use.

Protocol

1. Sample preparation

Serum, Plasma, or environment liquid samples can be measured directly, urine and saliva need a series of dilutions of the sample to ensure the readings are within the standard curve range. Homogenize Cell (2 x 10^6) or tissue (20 mg) sample in 200 µL cold PBS. Centrifuge to collect the supernatant. It is recommended with all sample types to assay immediately or aliquot and store the samples at -80°C.

Transfer 10 µL sample into the clear 96-well flat bottom plate in duplicate.

2. Standard Preparation

Transfer 20 µL 100 mM NH_4Cl standard into 380 µL dH_2O to generate a 5 mM NH_4Cl standard, then following the table to generate 5, 3, 1.5, 0.5 and 0 mM NH_4Cl standards.

NH_4Cl std(µL)	dH_2O(µL)	final NH_4Cl concentration (mM)
100	0	5
60	40	3
30	70	1.5
10	90	0.5
0	100	0

Transfer 10 µL of appropriate standards into the 96-well plate in duplicate. The blank control containing dH_2O only.

3. Reaction

Prepare enough working reagent by mixing 85 µL assay buffer, 4 µL Reagent A, 4 µL Reagent B for each reaction. Transfer 90 µL prepared working reagent into each standard and samples well. Tap plate to mix well. Incubate 20 min. at room temperature, protected from light.

4. Measurement

Read the optical density at 420 nm (400-440 nm).

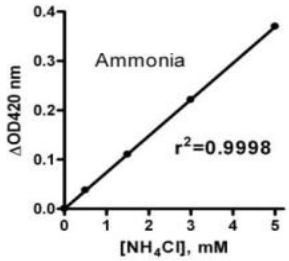
5. Calculation

Average the duplicate OD420 nm reading for standard and sample. Subtract the average OD of the blank from the average OD of the standards and plot the result (ΔOD) versus the ammonia concentration of the standards. Determine the slope and calculate the ammonia concentration of samples.

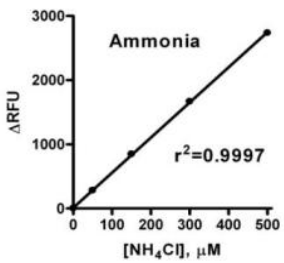
[Ammonia]= (OD_{sample}-OD_{blank})/Slope x *n* (mM)

OD_{sample} and OD_{blank} are related optical density of the sample and dH_2O. *n* is the sample dilution factor.

Typical Standard Curve



NH_4Cl standard in 96 wells-plate assay (colorimetric).



NH_4Cl standard in 96 wells-plate assay (fluorimetric).

Always run your own standard curves for calculation of results.

Fluorimetric assay is around 10-fold sensitivity than colorimetric assay.
Prepared ammonia standard from above should be 10-fold more dilute with dH_2O to generate 500, 300, 150, 50 and 0 µM NH_4Cl standards.

Transfer 10 µL of appropriate standards and samples into a 96-well black microplate in duplicate.

Prepare enough working reagent by mixing 85 µL assay buffer, 4 µL Reagent A, 4 µL Reagent B. Transfer 90 µL prepared working reagent into each reaction well. Tap plate to mix well. Incubate 20 min. at room temperature, protected from light.

Read fluorescence at Ex=410nm (400-440nm) and Em=470nm. Subtract the average RFU of the blank from the average RFU of the standards and plot the result (ΔRFU) versus the ammonia concentration of the standards. Determine the slope and calculate the ammonia concentration of samples. The ammonia concentration of sample is calculated as:

[Ammonia]= (RFU_{sample}-RFU_{blank})/Slope x *n* (µM)

RFU_{sample} and RFU_{blank} are related fluorescence units of the sample and dH_2O. *n* is the sample dilution factor.

Sensitivity and Limit of Detection

The Limit of Detection was determined as 0.018 mM, and linear detection range up to 5 mM in 96-well plate colorimetric assay; and 0.27-500 µM in 96-well plate fluorimetric assay. Sensitivity was determined as 0.1 µM. Samples with values above 5 mM (500 µM fluorimetric) should be dilute with dH_2O or assay buffer, re-assayed, and multiply results by dilution factor.

Interferences

The assay is highly specific for ammonia since amino acids, peptides, amines, and amides do not interfere.

References

Taylor, S. et al. 1974, Anal. Biochem. 60:153-162
Mroz, EA. et al. 1982, kidney International. 21:524-527
Roman, RJ. et al. 1979, Anal. Biochem. 98:136-141

Related Products:

ALP Colorimetric Detection Kit (#K109-200)
ALP Fluorimetric Detection Kit (#K110-200)
ALP Substrate Solution (#C8040)
BCIP/NBT Substrate system (#C8041)
Creatinine Detection Kit (#K148-200)
Glucose Detection Kit (#K188-100)
Glutathione Detection Kit (#K140-100)
Glutathione Peroxidase Detection Kit (#K143-100)
Glutathione Reductase Detection Kit (#K146-100)
HRP Fluorimetric Detection Kit (#K210-100)
HRP Substrate system (#C8042)
Xanthine Colorimetric Detection Kit (#K133-100)
SOD Activity Detection Kit (#K129-100)
Ammonia Colorimetric Detection Kit (#K203-100)
Sulfate Detection Kit (#K149-200)
Heme Detection Kit (#K169-200)
Lactate Detection Kit (#K207-100)
NAD/NADH Detection Kit (#K120-100)
NADP/NADPH Detection Kit (#K123-100)
Nitric Oxide Detection Kit (#K116-100)
Phosphate Detection Kit (#K198-500)
Urea Detection Kit (#K158-200)
pNPP substrate solution (#C8071)
TBARS Detection Kit (#K145-100)
Peroxidase Activity Detection Kit(#K126-200)
Hemoglobin Detection Kit (#K168-200)
Xanthine Oxidase Activity Detection Kit (#K132-100)
Lactate Dehydrogenase Activity Detection Kit (#K107-100)
Alcohol Dehydrogenase Activity Detection Kit (#K113-100)
Calcium Detection Kit (#K301-200)