Ammonia Assay (AA)

Cat. No. 8528
100 Tests in 96-well plate

Introduction

Ammonia is an important source of nitrogen for living systems. It is produced from dietary and amino acid metabolism and is toxic to the central nervous system (CNS) at high concentrations. Ammonia is taken up by the liver and converted to urea through the urea cycle. Blood ammonia measurement has been used in the diagnosis of comas associated with hepatic dysfunction caused by cirrhosis and neoplasms. This colorimetric assay is based on the oxidization of NADPH to NADP in the presence of α-ketoglutaric acid and glutamate dehydrogenase. The ammonia level is determined by assaying the rate of NADPH oxidation, which is proportional to the reduction in absorbance at 340 nm over time.

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8528a	1	Assay buffer	25 mL	4°C
8528b	1	Ammonia standard	1.0 mL	4°C
8528c	1	Cofactor	1 vial	-20°C
8528d	1	Substrate	200 µL	-20°C
8528e	1	Enzyme	100 µL	-20°C

Product Use

The Ammonia Assay kit measures the ammonia level of different types of samples, such as plasma, tissues and cell lysate. This product is for research purposes only and not for use in animals, humans, or diagnostic procedures.

Quality Control

Serially diluted ammonia solutions with concentrations ranging from 3.125 to 100 µg/mL are measured with the ScienCell™ Ammonia Assay kit. The change in OD_{340nm} is monitored as a function of time (Figure 1) and the resulting standard curve of ΔOD_{340nm}/min vs ammonia concentration are plotted (Figure 2). A positive linear relationship between ΔOD_{340nm}/min & ammonia concentration can be observed.

Shipping

Shipped on dry ice

Reagents Preparation

1. Working buffer: Add 167 µL substrate (8528d) into 10 mL assay buffer and then dissolve the contents of the bottle of cofactor (8528c) with them and keep at -20°C. Avoid freeze/thaw cycles.

Procedure (96-well plate)

A. Preparation of ammonia standard

1. Add 10 µL of ammonia standard (8528b) to 40 µL of assay buffer (8528a) to make a 50 µL solution of 200 µg/mL ammonia.
2. Obtain 7 test tubes and label them #1 through #7, add 40 µL of assay buffer into #1-7 tube.
3. Add 40 µL 200 µg/mL ammonia into tube #1, mix well in tube #1 to get the 100 µg/mL ammonia standard.
4. Transfer 40 µL of the 100 µg/mL ammonia standard from tube #1 to tube #2 and mix well to get the 50 µg/mL ammonia standard.
5. Repeat step 3 for tubes #3-6 to serially dilute the ammonia standards. Do not add any ammonia to tube #7, which serves as blank.
6. Obtain a 96-well test plate, prepare 2 replicates (A, B) of each ammonia standard by aliquoting 10 µL/well of each ammonia standard into duplicate wells of the 96-well test plate, according to the following plate format:

	#1	#2	#3	#4	#5	#6	#7
A	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.125 µg/ml	Blank
B	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.125 µg/ml	Blank

B. Preparation of test samples

1. Cell pellet or tissue can be homogenized in 4 volumes of the assay buffer. Centrifuge the samples at 10,000 ×g for 10 minutes at 4ºC to remove insoluble material. The soluble clear fraction can be assayed directly.
2. Samples should be serial diluted to make sure the readings are within the standard curve range. Prepare test samples to a final volume of 10 µL/well on the 96-well flat bottom plate.

C. Working reagent preparation and measurements

1. For each well of reaction, prepare working reagent by mixing 89 µL working buffer and 1 µL enzyme (8528e).
2. Add 90 µL of working reagent mix into each well of the 96-well plate containing ammonia standard, samples and blank. Start recording OD_{340nm} over a 3 minutes interval, collecting data every 0.5 minute.

D. Calculations

1. Subtract the measured OD_{340nm} at different reaction time from the initial OD_{340nm} to obtain the corresponding ΔOD_{340nm} for each sample and ammonia standard at different reaction time. Average the value of ΔOD_{340nm} of replicate wells. Subtract the measured ΔOD_{340nm} at different reaction time from the blank.
2. Based on the ΔOD_{340nm} of the ammonia standard solutions, plotting the absorbance at ΔOD_{340nm} as a function

of reaction time (Figure 1) in which ΔOD_{340nm} /min is calculated.

3. Plot a standard curve of ΔOD_{340nm} /min vs ammonia concentration (Figure 2).
4. Calculate the ammonia concentration of test samples based on the standard curve.

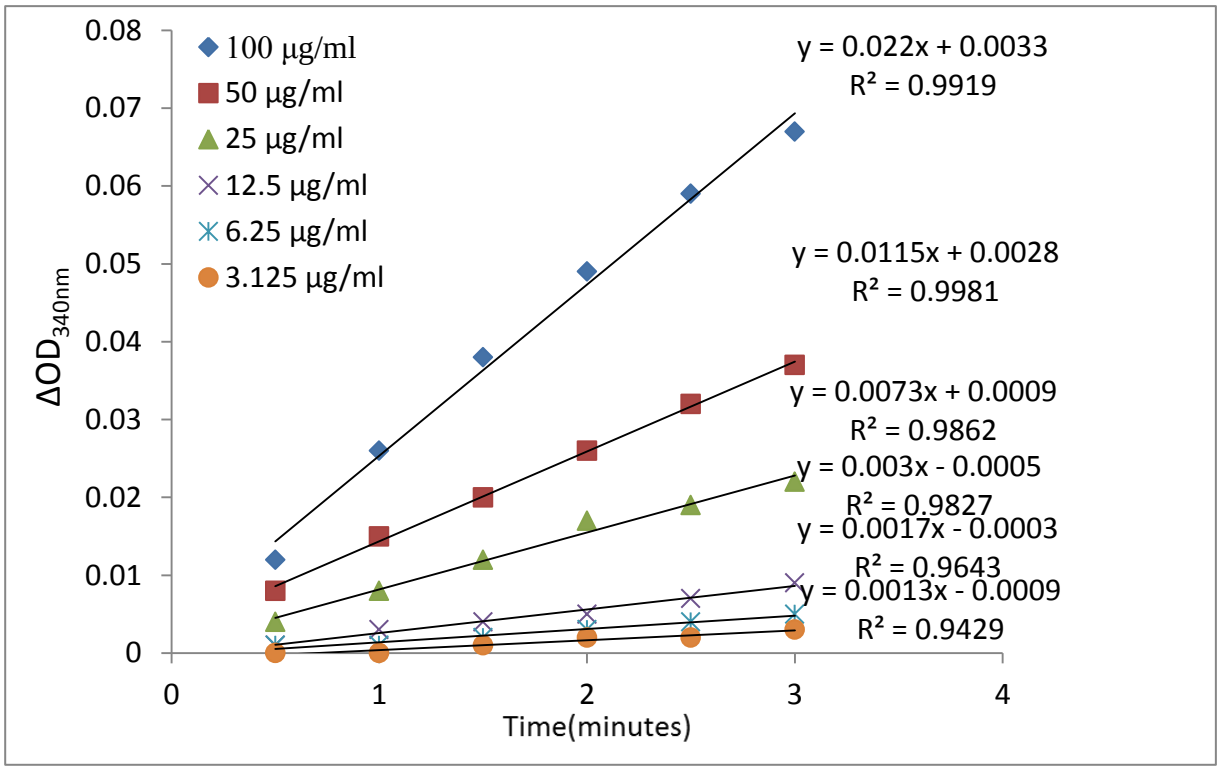

Figure1. Standard curves of ΔOD_{340nm} vs reaction time for ammonia solution with different concentration.

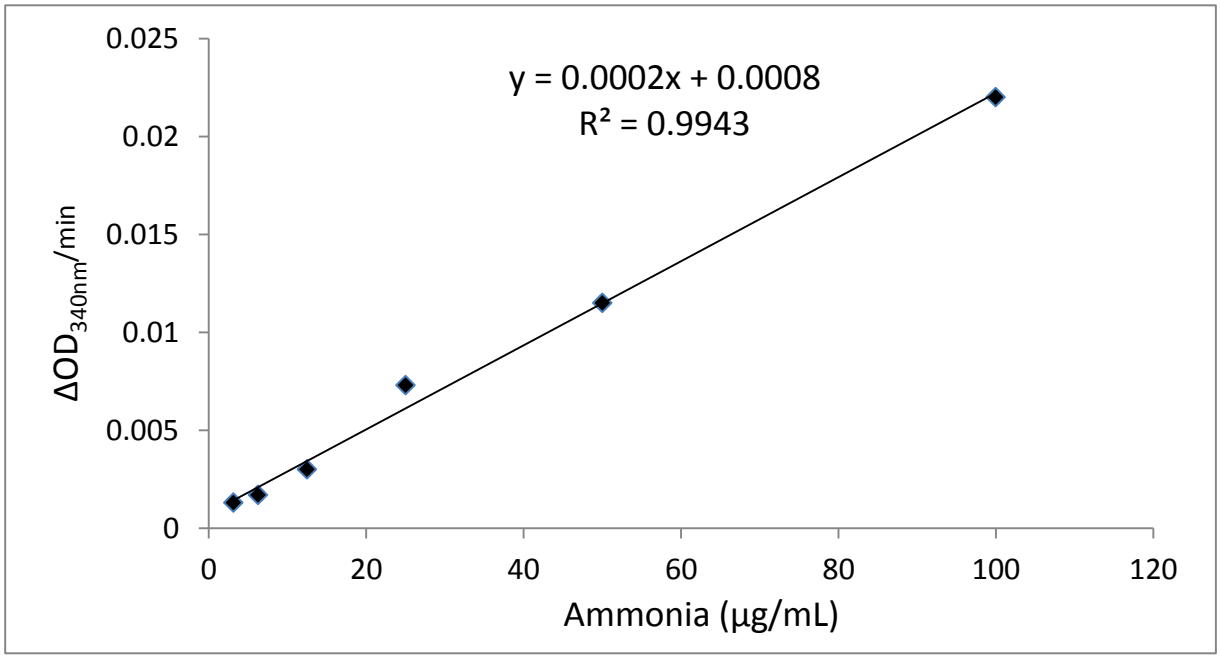

Figure2. Standard curve of ΔOD_{340nm}/min vs concentration of ammonia. The ΔOD_{340nm}/min is calculated as the slope of the standard curves shown in Figure 1.