Blood Ammonia Colorimetric Assay Kit

Catalog No: E-BC-K145-S

Method: Colorimetric method

Specification: 100 Assays (Can detect 96 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 0.01 mmol/L

Detection range: 0.01-2.0 mmol/L

- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

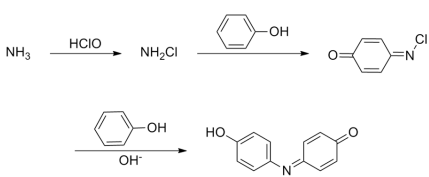
This kit can be used for detection of blood ammonia content in serum or plasma samples.

▲Background

NH_3 or $NH4^+$ is an important source of nitrogen in biological systems. Ammonia is a metabolite produced by amino acid deamination. In living systems, glutamate dehydrogenase and glutamine synthase are key regulators of amino acid and ammonia metabolism. Glutamate dehydrogenase catalyzes the oxidation of glutamate to α-ketoglutaric acid and ammonia, while glutamine synthase is used to eliminate excess ammonia.

▲Detection principle

Blood protein can be precipitated with protein precipitator, and enzyme activity will be destroyed, which can prevent the formation of free ammonia in vitro. Most interfering color substances were removed at the same time, indigo was formed in non-protein filtrate by Berthelot reaction, and the color depth was proportional to the content of blood ammonia. Blood ammonia content can be determined by comparing with standard solution.



Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Acid Reagent	35 mL × 2 vials	2~8℃ , 3 months
Reagent 2	Chromogenic Agent A	60 mL × 2 vials	2~8℃ ,3 months, shading light
Reagent 3	Chromogenic Agent B	60 mL × 2 vials	2~8℃ , 3 months, shading light
Reagent 4	7 mmol/L Ammonia Standard	2 mL × 1 vial	2~8℃ ,3 months
Reagent 5	Standard Diluent	50 mL × 1 vial	2~8℃ ,3 months
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

Materials prepared by users

Instruments

Spectrophotometer(635 nm), Micropipettor, Vortex mixer, Incubator, Centrifuge

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL, 5 mL)

Reagents

Double distilled water, Normal saline (0.9% NaCl)

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲The key points of the assay

1. The supernatant after centrifugation of protein precipitation must be clarified, and the following step should be conducted within 20 min.
2. Reagent 2 and reagent 3 cannot be mixed before use.
3. The detection of OD value should be completed within 20 min.

Pre-assay preparation

▲Reagent preparation

Preparation of 0.35 mmol/L standard working solution:

Dilute the reagent 4 with reagent 5 at the ratio of 1:19 and mix fully.

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

Sample requirements

1. The amount of ammonia in the red blood cells is 2.8 times higher than that in the plasma, so the sample should avoid hemolysis, or the result will be influenced.

2. Since glutamine and polypeptides in blood samples are easy to release ammonia by water interpretation, the samples should be timely tested. The samples can be stored sealed at 2-8°C for 2-4 h, and at -20°C for 24 h.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.01-2.0 mmol/L).

The recommended dilution factor for different samples is as follows (for reference only)

Sample type	Dilution factor
Human serum	2-6
Rat serum	1
Rabbit serum	1
Mouse serum	1

Note: The diluent is double distilled water or normal saline (0.9% NaCl).

Instructions for the use of transferpettor

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Operating steps

(1) Blank tube: Take 0.2 mL of reagent 5 to the 1.5 mL EP tube.
Standard tube: Take 0.2 mL of 0.35 mmol/L standard working solution to the 1.5 mL EP tube.

Sample tube: Take 0.2 mL of sample to the 1.5 mL EP tube.

(2) Add 0.6 mL of reagent 1 and mix fully with a vortex mixer and centrifuge at 1100 g for 10 min.
Note: the following step (chromogenic reaction) should be conducted within 20 min.

(3) Take 0.4 mL of supernatant from each tube of Step 2 into corresponding tubes.

(4) Add 1.0 mL of reagent 2 and 1.0 mL of reagent 3 sequentially into each tube of Step 3. Mix fully with a vortex mixer.
Note: Reagent 2 and reagent 3 cannot be mixed before use.

(5) Incubate the tubes in 37℃ water bath for 30 min. Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 635 nm with 1 cm optical path quartz cuvette.
Note: the detection of OD value should be completed within 20 min.

▲Operation table

1. Pretreatment of samples

	Blank tube	Standard tube	Sample tube
Reagent 5 (mL)	0.2		
0.35 mmol/L standard working solution (mL)		0.2	
Sample (mL)			0.2
Reagent 1	0.6	0.6	0.6
Mix fully with a vortex mixer and centrifuge at 1100 g for 10 min, then take the supernatant for chromogenic reaction.			

2. Chromogenic reaction

	Blank tube	Standard tube	Sample tube
Supernatant (mL)	0.4	0.4	0.4
Reagent 2 (mL)	1.0	1.0	1.0
Reagent 3 (mL)	1.0	1.0	1.0
Mix fully with a vortex mixer and incubate at 37℃ for 30 min. Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 635 nm with 1 cm optical path quartz cuvette.			

▲Calculation

Serum sample:

$$\text{Blood ammonia content (mmol/L)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f$$

Note:

ΔA_1: $OD_{Sample} - OD_{Blank}$

ΔA_2: $OD_{Standard} - OD_{Blank}$

c: Concentration of standard, 0.35 mmol/L

f: Dilution factor of sample before test.

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 3 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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Detection range	0.01-2.0 mmol/L	Average intra-assay CV (%)	4.7
Sensitivity	0.01 mmol/L	Average inter-assay CV (%)	5.0
Average recovery rate (%)	104		

▲Example analysis

Take 0.2 mL of human serum (diluted for 4 times), carry the assay according to the operation table. The results are as follows:

The average OD value of the sample is 0.032, the average OD value of the blank is 0.008, the average OD value of the standard is 0.105, the concentration of the standard is 0.35 mmol/L, and the calculation result is:

Blood ammonia content(mmol/L)=(0.032-0.008)÷(0.105-0.008)×0.35×4
=0.346 (mmol/L)

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -20℃ for 24 h.

▲ Plasma

Take fresh blood into the tube which has anticoagulant, centrifuge at 700-1000 g for 10 min at 4℃ . Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -20℃ for 24 h.

▲ Notes for sample

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
2. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.