Blood Ammonia Colorimetric Assay Kit

Catalog No: E-BC-K145-M

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 0.01 mmol/L

Detection range: 0.01-2.5 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

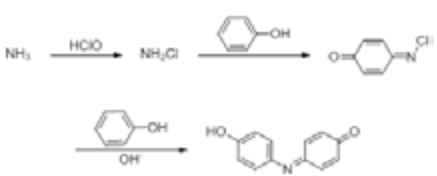
This kit can measure blood ammonia content in serum and plasma samples.

▲Background

Ammonia (NH_3) or its ionic form is an important source of nitrogen in biological systems. Ammonia is a metabolite produced by the deamination of amino acids. In living systems, glutamic acid dehydrogenase and glutamine synthetase are key regulators of amino acid and ammonia metabolism. Glutamic acid dehydrogenase catalyzes the oxidation of glutamic acid to α-ketoglutarate and ammonia, and glutamic acid Aminamide 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	40 mL × 1 vial	2-8℃ , 3 months
Reagent 2	Chromogenic Agent A	20 mL × 1 vial	2-8℃ , 3 months, shading light
Reagent 3	Chromogenic Agent B	20 mL × 1 vial	2-8℃ , 3 months, shading light
Reagent 4	7 mmol/L Standard	1.5 mL × 1 vial	2-8℃ , 3 months
Reagent 5	Standard Diluent	30 mL × 1 vial	2-8℃ , 3 months
	Microplate	96 wells	
	Plate Sealer	2 pieces	
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

Microplate reader (600-660nm), Micropipettor, Centrifuge, Incubator, Vortex mixer

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 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 must be clarified and the chromogenic reaction must be carry out in 20 min.
2. Reagent 2 and reagent 3 can't be mixed before adding.
3. It is recommended to use disposable material to avoid the contamination of interfering substances.

Pre-assay preparation

▲Serum sample

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.

▲Plasma sample

The fresh blood was added into the test tube containing anticoagulant and mixed upside down. Centrifuge the sample at 4℃ for 10 min at 700~1000 g, the upper yellowish transparent liquid was taken as the plasma, and the middle white interference layer (white blood cells and platelets) could not be absorbed. Place the plasma on ice for detection.

[Sample requirements]:

a) The ammonia content of red blood cells is 2.8 times higher than that of plasma, so samples need to avoid hemolysis when testing, to prevent ammonia in red blood cells from entering the plasma.

b) Because the glutamine and peptides are easily hydrolyzed and release ammonia, the samples should be tested in time. The sample can be stored at 2-8℃ for 2-4 hours or at -20℃ for 24 hours.

c) Seal immediately after sampling to avoid ammonia spillage.

▲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 (0.01-2.5 mmol/L).

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

Sample type	Dilution factor
Human serum	1
Human plasma	1
Mouse serum	1
Rat plasma	1
Dog serum	1
Horse serum	1

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

Assay protocol	
Ambient temperature	25-30℃
Optimum detection wavelength	635nm

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

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

[Note]: A-H, standard wells; S1-S80, sample wells.

▲Operating steps

1. The preparation of standard curve

Dilute 7 mmol/L standard with reagent 5 to a serial concentration. The recommended dilution gradient is as follows: 0, 0.2, 0.4, 0.8, 1.0, 1.5, 2, 2.5 mmol/L.

2. The measurement of samples

1) Standard tube: Add 100 μL of standard solution with different concentrations to the 1.5 mL EP tube.
   Sample tube: Add 100 μL of sample to the 1.5 mL EP tube.
2) Add 300 μL of reagent 1, mix fully with vortex mixer. Centrifuge the tubes at 1100 g for 10 min.
   Note: the following steps (chromogenic reaction) must be carry out in 20 min.
3) Take 40 μL of supernatant of each tube to the corresponding well of microplate.
4) Add 120 μL of reagent 2 and 120 μL of reagent 3 successively (Reagent 2 and reagent 3 can't be mixed before adding).
5) Mix fully with microplate reader for 5 s, incubate at 37℃ for 25 min.
6) Measure the OD value of each well with microplate reader at 635 nm.

▲Operation table

	Standard tube	Sample tube
Standard solution with different concentrations (μL)	100	
Sample (μL)		100
Reagent 1 (μL)	300	300
Mix fully with vortex mixer. Centrifuge the tubes at 1100 g for 10 min, then take the supernatant for the following steps in 20 min.		
Supernatant (μL)	40	40
Reagent 2 (μL)	120	120
Reagent 3 (μL)	120	120
Mix fully with microplate reader for 5 s, incubate at 37℃ for 25 min. Measure the OD value of each well with microplate reader at 635 nm.		

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

$$\text{Blood ammonia (mmol/L)} = (\Delta A_{635} - b) \div a \times f$$

Note:

y: $OD_{Standard} - OD_{Blank}$.

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

f: Dilution factor of sample before tested.

ΔA_{635}: $OD_{Sample} - OD_{Blank}$.

▲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	0.01-2.5 mmol/L	Average intra-assay CV (%)	4.1
Sensitivity	0.01 mmol/L	Average inter-assay CV (%)	7.2
Average recovery rate (%)	103		

▲Example analysis

Take 100 μL of human serum and carry the assay according to the operation table. The results are as follows:

Standard curve: y = 0.3198 x – 0.0124, the average OD value of the sample is 0.314, the average OD value of the blank is 0.050, and the calculation result is:

$$\text{Blood ammonia (mmol/L)} = \frac{0.314 - 0.050 + 0.0124}{0.3198} = 0.86 \text{ mmol/L}$$