（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K145-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（600-660 nm）

Detection range：0.01-2.5 mmol/L

Elabscience® Blood Ammonia Colorimetric Assay Kit

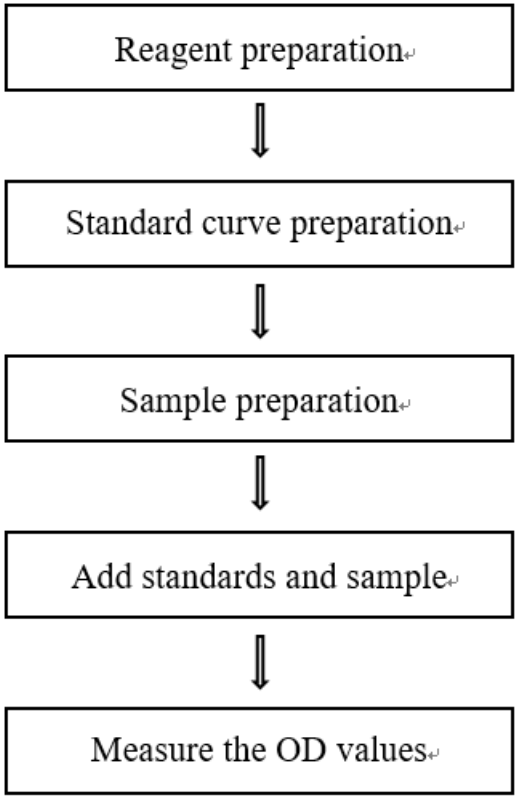
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

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

Table of contents

Assay summary

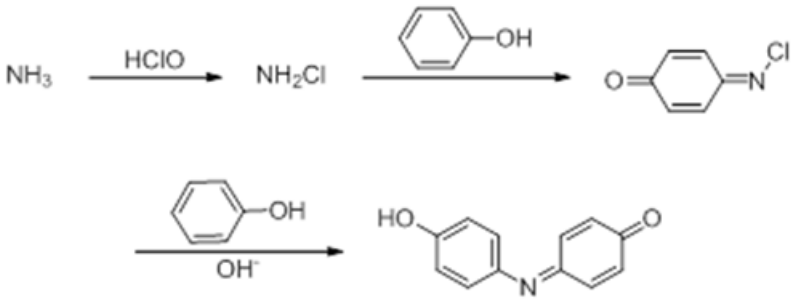


Intended use

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

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	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Acid Reagent	20 mL ×1 vial	40 mL ×1 vial	2-8℃, 12 months
Reagent 2	Chromogenic Agent A	10 mL ×1 vial	20 mL ×1 vial	2-8℃, 12 months, shading light
Reagent 3	Chromogenic Agent B	10 mL ×1 vial	20 mL ×1 vial	2-8℃, 12 months, shading light
Reagent 4	7 mmol/L Standard	1.5 mL ×1 vial	1.5 mL ×1 vial	2-8℃, 12 months
Reagent 5	Standard Diluent	30 mL ×1 vial	30 mL ×1 vial	2-8℃, 12 months
	Microplate	96 wells		No requirement
	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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (600-660 nm, optimum wavelength: 635 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 7 mmol/L zinc standard with standard diluent 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. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.2**	**0.4**	**0.8**	**1.0**	**1.5**	**2.0**	**2.5**
7 mmol/L standard (μL)	0	10	20	40	50	75	100	125
Standard diluent (μL)	350	340	330	310	300	275	250	225

Sample preparation

① Sample preparation

Serum and plasma: detect directly.

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.

② Dilution of sample

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). For the dilution of other sample types, please do pretest to confirm the dilution factor.

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 Chromogenic agent A and chromogenic agent B can't be mixed before adding.

③ It is recommended to use disposable material to avoid the contamination of interfering substances.

Operating steps

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 acid reagent, 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.

④ Add 120 μL of chromogenic agent A and 120 μL of chromogenic agent B successively (chromogenic agent A and chromogenic agent B 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.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

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

[Note]

f: Dilution factor of sample before tested.

ΔA_{635}: $OD_{Sample} - OD_{Blank}$.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.45	1.26	2.00
%CV	4.2	4.3	3.8

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.45	1.26	2.00
%CV	7.1	7.5	7.0

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 100%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.35	0.88	1.7
Observed Conc. (mmol/L)	0.4	0.9	1.8
Recovery rate (%)	101	104	104

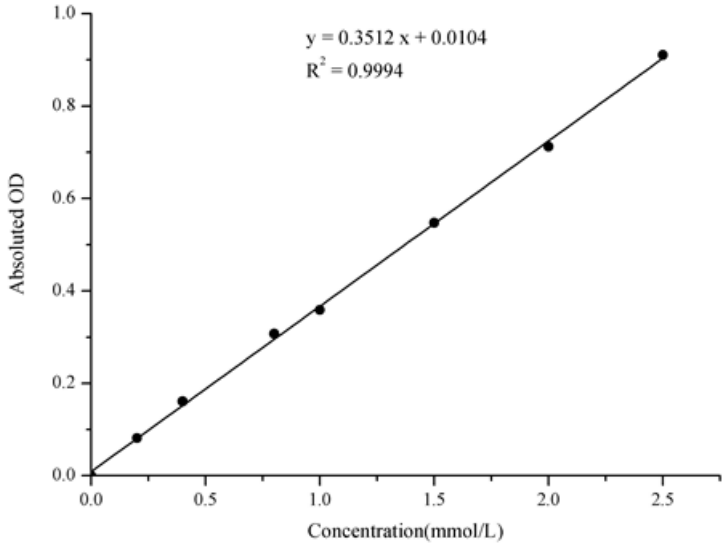
Sensitivity

The analytical sensitivity of the assay is 0.01 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (mmol/L)	0	0.2	0.4	0.8	1.0	1.5	2	2.5
Average OD	0.054	0.135	0.215	0.361	0.413	0.601	0.767	0.965
Absoluted OD	0	0.081	0.161	0.307	0.359	0.547	0.713	0.911



Appendix II Example Analysis

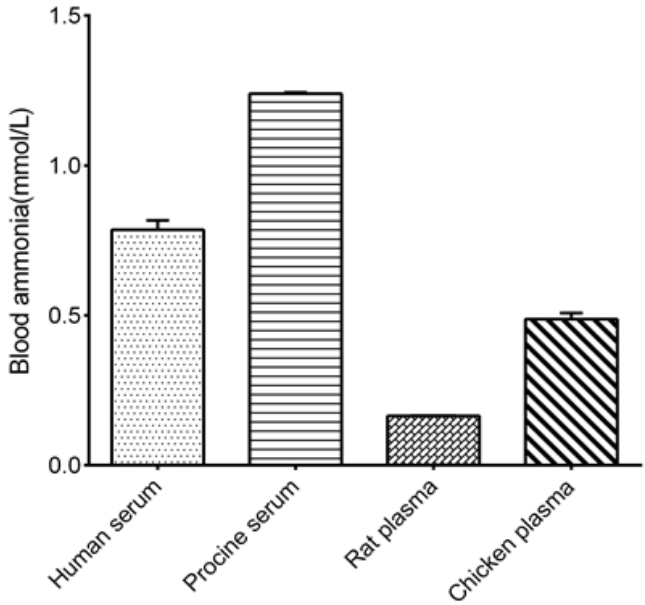
Example analysis:

Take 100 μL of human serum and carry the assay according to the operation steps. 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:

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

Detect human serum, porcine serum, rat plasma and chicken plasma according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.