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

Catalog No：E-BC-K145-S

Specification：50 assays(48 samples)/ 100 assays(96 samples)

Measuring instrument：Spectrophotometer（635 nm）

Detection range：0.01-2.0 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.

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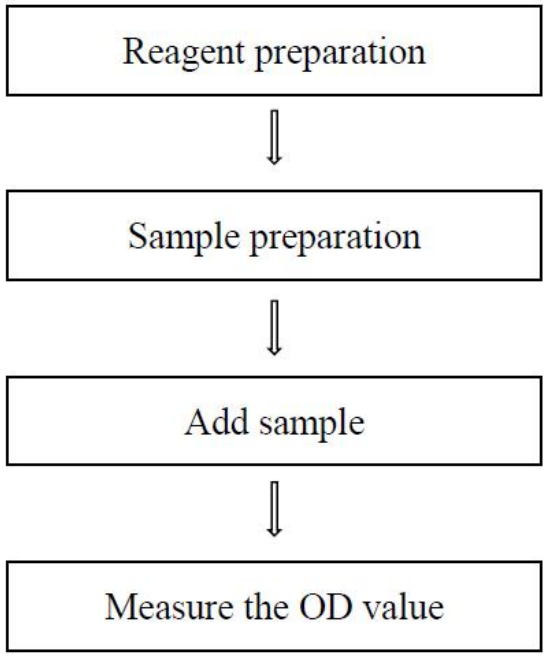
Assay summary

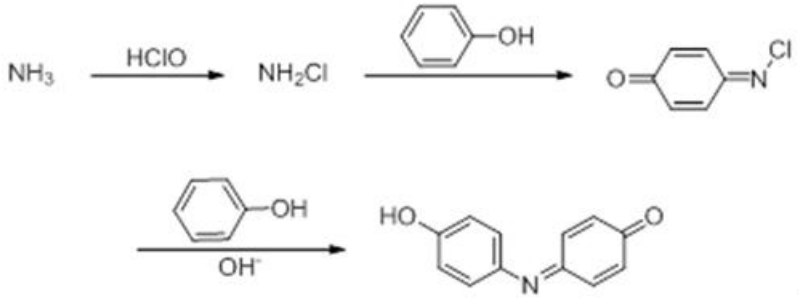


Intended use

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 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Acid Reagent	35 mL × 1 vial	35 mL × 2 vials	2-8°C, 12 months
Reagent 2	Chromogenic Agent A	60 mL ×1 vial	60 mL ×2 vials	2-8°C, 12 months, shading light
Reagent 3	Chromogenic Agent B	60 mL × 1 vial	60 mL × 2 vials	2-8°C, 12 months, shading light
Reagent 4	7 mmol/L Ammonia Standard	2 mL × 1 vial	2 mL × 1 vial	2-8°C, 12 months
Reagent 5	Standard Diluent	50 mL × 1 vial	50 mL × 1 vial	2-8°C, 12 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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

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 0.35 mmol/L standard working solution：

For each well, prepare 200 μL of 0.35 mmol/L standard working solution (mix well 10 μL of 7 mmol/L ammonia standard and 190 μL of standard diluent).

Sample preparation

① Sample preparation

Serum and plasma: detect directly.

Sample requirements:

a) 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.

b) 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.

② Dilution of sample

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). 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 The detection of OD value should be completed within 20 min.

Operating steps

① Blank tube: Take 0.2 mL of standard diluent 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 acid reagent 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 chromogenic agent A and 1.0 mL of chromogenic agent B sequentially into each tube of Step 3. Mix fully with a vortex mixer.
Note: Chromogenic agent A and chromogenic agent B cannot be mixed before use.

⑤ Incubate the tubes in 37°C 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.

Calculation

The 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.

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)	0.35	1.05	1.60
%CV	5.0	4.6	4.5

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)	0.35	1.05	1.60
%CV	4.8	5.1	5.1

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 104%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.65	1.2	1.7
Observed Conc. (mmol/L)	0.7	1.2	1.8
Recovery rate (%)	105	103	104

Sensitivity

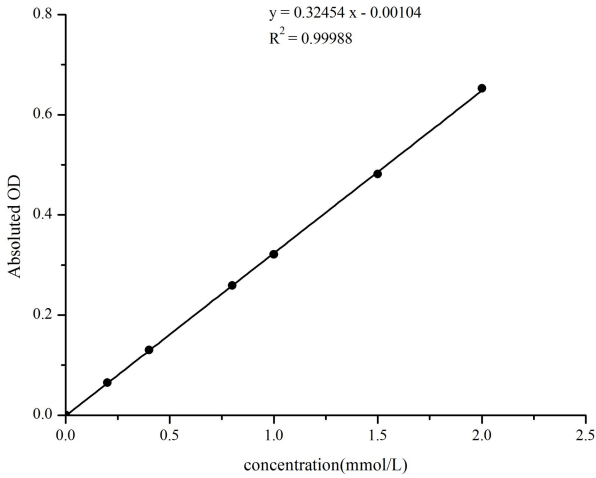
The analytical sensitivity of the assay is 0.01 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

(It doesn't need to prepare the standard curve for this kit and the provided standard curve is for reference only)

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	0
Average OD	0.010	0.075	0.140	0.269	0.332	0.492	0.663	0.010
Absoluted OD	0	0.065	0.130	0.259	0.322	0.482	0.653	0



Appendix II Example Analysis

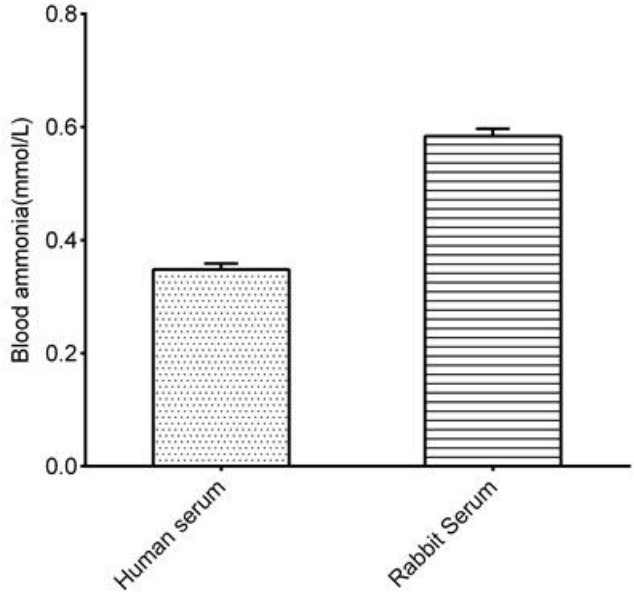
Example analysis：

Take 0.2 mL of human serum (diluted for 4 times), carry the assay according to the operation steps. 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:

$$\text{Blood ammonia content (mmol/L)} = \frac{0.032 - 0.008}{0.105 - 0.008} \times 0.35 \times 4 = 0.346 \text{ (mmol/L)}$$

Detect human serum (dilute for 4 times), rabbit serum 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.