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

Blood Ammonia Assay Kit

Catalog No.: BC00050

Size: 100T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Blood Ammonia Assay Kit
Detection Method	Colorimetric
Sample Type	Serum, plasma and other samples
Assay Type	Quantitative
Detection Instrument	Microplate reader (635 nm)

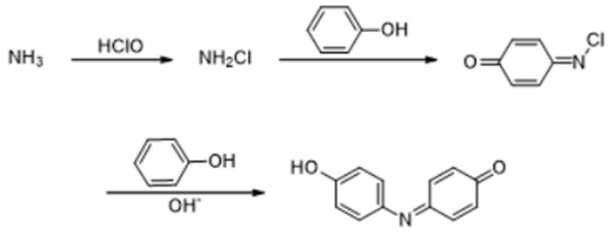
Product Introduction

The main sources of blood ammonia are endogenous ammonia and exogenous ammonia. Ammonia maintains a constant state in the blood, that is, the source and destination of blood ammonia maintain a dynamic balance. Ammonia is a toxic substance and is mainly metabolized and detoxified in the liver. When liver function is severely damaged, ammonia cannot be detoxified. Ammonia accumulates in the central nervous system, leading to hepatic encephalopathy.

Principle

This method is based on the principle of ammonia indophenol blue reaction. After precipitating the protein in serum (plasma) with a protein precipitant, the blood ammonia is determined using the phenol-hypochlorite direct colorimetric method. The generated blue indophenol is proportional to the concentration of ammonia and has a special absorption peak at 635nm. Based on this, the blood ammonia content in the sample can be calculated from the absorbance value.

Use protein precipitant to precipitate blood protein and destroy enzyme activity to prevent free ammonia from being produced after being separated from the body. At the same time, remove most of the interfering color substances. Use Berthelot reaction to form indigo in the protein-free filtrate. The color depth is proportional to the blood ammonia content. Compare with the standard solution to determine the blood ammonia content. The detection principle is as follows:



Components

No.	Components	Size (100T)	Storage
Reagent 1	Protein Precipitant I	20 mL	-20℃, store at 2-8°C after opening.
Reagent 2	Protein Precipitant Ⅱ	20 mL	-20℃, store at 2-8°C after opening.
Reagent 3	Color Developer I	15 mL	-20℃, protect from light, store at 2-8°C after opening.
Reagent 4	Color Developer II	15 mL	-20℃, protect from light, store at 2-8°C after opening.
Reagent 5	7mmol/L Standard	1.5 mL	-20℃, store at 2-8°C after opening.
Consumable 1	Microplate	1 plate	RT
Consumable 2	Plate Sealer	2 pieces	RT

Storage

The unopened kit can be stored at -20℃ for 12 months, and after opening, it can be stored at 2-8℃ for 3 months.

Preparation

- **Sample handling**

1. Serum samples: Can be measured directly.

2. Sample dilution: Generally, serum samples do not need to be diluted. Before the formal test of special samples, 2-3 samples with large expected differences can be selected and diluted into different concentrations for preliminary experiments. According to the results of the preliminary experiments, combined with the linear range of this kit: 0.01-4mmol/L, dilution is performed. The diluent is double distilled water or physiological saline (0.9 % NaCl).

- **Preparation of the kit**

1. Take out all reagents and return to room temperature before use.
2. Prepare different concentrations of standards by diluting the standard solution with double-distilled water to 4mmol/L or 2mmol/L, then dilute to different concentrations such as 4, 2, 1, 0.5, 0.25, 0.125, 0 (blank well) mmol/L using the halving dilution method.

Operation process

1. Standard tube: Take 100 μL of each concentration of standard solution and add to 1.5 mL EP tube; Measurement tube: 100 μL of sample to be tested, added to 1.5 mL EP tube.
2. Add 150 μL of Reagent 1 and 150 μL of Reagent 2 to each tube from step (1), vortex mix, centrifuge at 1100×g for 10 min. (The color development must be carried out within 20 min after centrifugation).
3. Take 40 μL of the supernatant from each tube in step (2) and add to the wells of the plate.
4. Add 120 μL of Reagent 3 and 120 μL of Reagent 4 to each well of the plate from step (3) (Reagents 3 and 4 should not be mixed and used together).
5. Shake on the microplate reader for 5 seconds, incubate at 37℃ for 25 minutes, and measure the OD values at 635 nm with the microplate reader.

The operation table is as follows:

	Standard Tube (Well)	**Measurement Tube (Well)**
Application solution of each concentration standard (μL)	100	--
Sample (μL)	--	100
Reagent 1 (μL)	150	150

Reagent 2 (μL)	150	150
Vortex to mix, centrifuge at 1100 × g for 10 min, and add the supernatant to the ELISA plate.		
Supernatant (μL)	40	40
Reagent 3 (μL)	120	120
Reagent 4 (μL)	120	120
Shake on the plate reader for 5 seconds, incubate at 37℃ for 25 minutes, and measure OD values at 635 nm.		

Calculation

Standard fitting curve: y = ax + b

The calculation formula for blood ammonia content in serum, plasma and other liquids is:

Blood ammonia content (mmol/L) = (ΔA_{635} - b) ÷ a × f

y: Standard OD value - blank OD value (OD value when the standard concentration is 0)

x: concentration corresponding to the absorbance

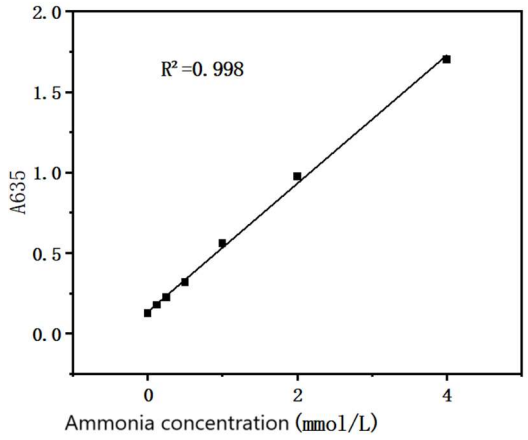
a: Slope of the standard curve

b: Intercept of the standard curve

ΔA_{635}: Sample OD value - Blank OD value

f: dilution factor of the sample before adding it to the detection system

The following standard curve is for reference only:



Notes

1. The ammonia content in red blood cells is 2.8 times higher than that in plasma, so **during testing, the sample must avoid hemolysis** to prevent the ammonia in the red blood cells from entering the plasma.
2. Since glutamine and polypeptides are easily hydrolyzed to release ammonia after blood samples are removed from the body, samples must be tested promptly after sampling and stored at 2-8℃ for 2-4 h or at -20℃ for 24 h.
3. After sampling, seal the container in time to avoid ammonia spillage.
4. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.