Technical Manual

Blood Ammonia Colorimetric Assay Kit

- **Catalogue Code: MAES0116**
- **Size: 96T**
- **Research Use Only**

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.01-2.5 mmol/L

Sensitivity:

0.01 mmol/L

Storage:

2-8°C for 3 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 3 months.

Do not use components from different batches of kit.

2. 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.

3. Intended Use

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

4. 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.

5. Kit components & storage

Item	Specification	Storage
Acid Reagent	40 mL × 1 vial	2-8°C, 3 months
Chromogenic Agent A	20 mL × 1 vial	2-8°C, 3 months, avoid direct sunlight
Chromogenic Agent B	20 mL × 1 vial	2-8°C, 3 months, avoid direct sunlight
Standard (7 mmol/L)	1.5 mL × 1 vial	2-8°C, 3 months
Standard Diluent	30 mL × 1 vial	2-8°C, 3 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (600-660 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)

6. Assay Notes:

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 prevent the contamination of interfering substances.

7. Reagent preparation:

Bring all reagents to room temperature before use.

8. Sample Preparation

Sample requirements:

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

2. 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°C for 2-4 hours or at -20°C for 24 hours.

3. Seal immediately after sampling to prevent ammonia spillage.

1. Serum sample:
Collect fresh blood and stand at 25°C for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection.

2. Plasma sample:
The fresh blood was added into the test tube containing anticoagulant and mixed upside down. Centrifuge the sample at 4°C 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 Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. 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).

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 635 nm

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.

10. Operation Steps

The preparation of standard curve

Dilute standard (7 mmol/L) 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.

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 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.

4. Take 40 μL of supernatant of each tube to the corresponding well of microplate.

5. 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).

6. Mix fully with microplate reader for 5 s, incubate at 37°C for 25 min.

7. 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
Acid reagent (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
Chromogenic agent A (μL)	120	120
Chromogenic agent B(μL)	120	120
Mix fully with microplate reader for 5 s, incubate at 37°C for 25 min. Measure the OD value of each well with microplate reader at 635 nm.		

11. Calculations

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.

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

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}: $OD_{Sample} - OD_{Blank}$.

12. Performance Characteristics

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

Analysis

Take 100 µL of human serum, 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:

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

Safety Notes

Some of the reagents in the kit contain dangerous substances. Avoid touching 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.

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

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