TECHNICAL MANUAL

Blood Ammonia Colorimetric Assay Kit

- **SKU CODE:** MAES0116
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

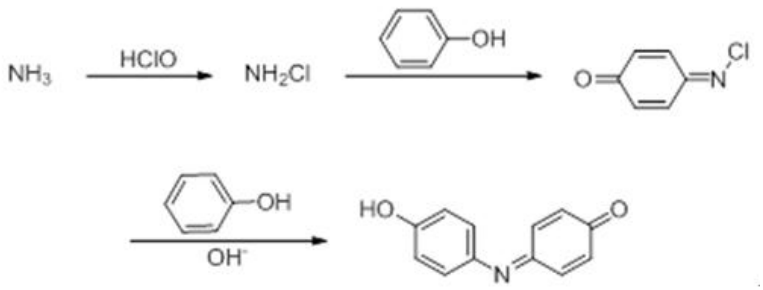
- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Centrifuge and take supernatant
- Add chromogenic agents
- Incubate and measure OD value

2. Intended use

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

3. Detection principle

Blood protein can be precipitated with protein precipitator, and enzyme activity is destroyed, which prevents the formation of free ammonia in vitro. Most interfering color substances are removed simultaneously. Indigo is formed in the non-protein filtrate by the Berthelot reaction, and the color intensity is proportional to the blood ammonia content. Blood ammonia content can be determined by comparing with standard solution.



4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Size 3(500Assays)	Storage
Reagent 1	Acid Reagent	20 mL × 1 vial	40 mL × 1 vial	40 mL × 5 vials	2-8°C, 12 months

Item	Component	Size 1(48 T)	Size 2(96 T)	Size 3(500Assays)	Storage
Reagent 2	Chromogenic Agent A	10 mL × 1 vial	20 mL × 1 vial	20 mL × 5 vials	2-8°C, 12 months, protect from light
Reagent 3	Chromogenic Agent B	10 mL × 1 vial	20 mL × 1 vial	20 mL × 5 vials	2-8°C, 12 months, protect from light
Reagent 4	7 mmol/L Standard	1.5 mL × 1 vial	1.5 mL × 1 vial	1.5 mL × 5 vials	2-8°C, 12 months
Reagent 5	Standard Diluent	30 mL × 1 vial	30 mL × 1 vial	30 mL × 5 vials	2-8°C, 12 months
	Microplate	48 wells	96 wells	/	No requirement
	Plate Sealer	2 pieces			

5. 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)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 7 mmol/L standard with standard diluent to create a serial concentration. The recommended dilution gradient is as follows: 0, 0.2, 0.4, 0.8, 1.0, 1.5, 2.0, 2.5 mmol/L.

7. Standard dilution table

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

8. 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 during testing to prevent ammonia from red blood cells entering the plasma. b) Because glutamine and peptides are easily hydrolyzed and release ammonia, samples should be tested promptly. Samples can be stored at 2-8°C for 2-4 hours or at -20°C for 24 hours. c) Seal immediately after sampling to avoid ammonia spillage.
2. **Sample dilution:** The recommended dilution factor for different samples is as follows (for reference only): 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 dilution of other sample types, please perform a pretest to confirm the dilution factor.

9. Key points of the assay

1. The supernatant after centrifugation must be clear, and the chromogenic reaction must be performed within 20 minutes.
2. Chromogenic agent A and chromogenic agent B cannot be mixed before adding.
3. It is recommended to use disposable materials to avoid contamination by interfering substances.

10. Operating steps

1. Standard tube: Add 100 µL of standard solution with different concentrations to 1.5 mL EP tubes. Sample tube: Add 100 µL of sample to 1.5 mL EP tubes.
2. Add 300 µL of acid reagent, mix thoroughly with vortex mixer. Centrifuge the tubes at 1100×g for 10 minutes. Note: The following steps (chromogenic reaction) must be performed within 20 minutes.
3. Take 40 µL of supernatant from each tube and add 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 cannot be mixed before adding).
5. Mix thoroughly with microplate reader for 5 seconds, incubate at 37°C for 25 minutes.
6. Measure the OD value of each well with microplate reader at 635 nm.

11. Calculation

The standard curve:

1. Average the duplicate readings for each standard.

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

3. Plot the standard curve using absolute OD value of standards and corresponding concentrations as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

The sample:

Blood ammonia (mmol/L) = (ΔA_635 - b) ÷ a × f

[Note]

f: Dilution factor of sample before testing.

ΔA_635: OD_{Sample} – OD_{Blank}.

12. Appendix I Performance Characteristics

Parameters:

Intra-assay Precision

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

Intra-assay Precision Results

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.

Inter-assay Precision Results

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

Recovery

Three samples of high, medium, and low concentrations were tested in 6 parallel replicates for each concentration to obtain an average recovery rate of 100%.

Recovery Results

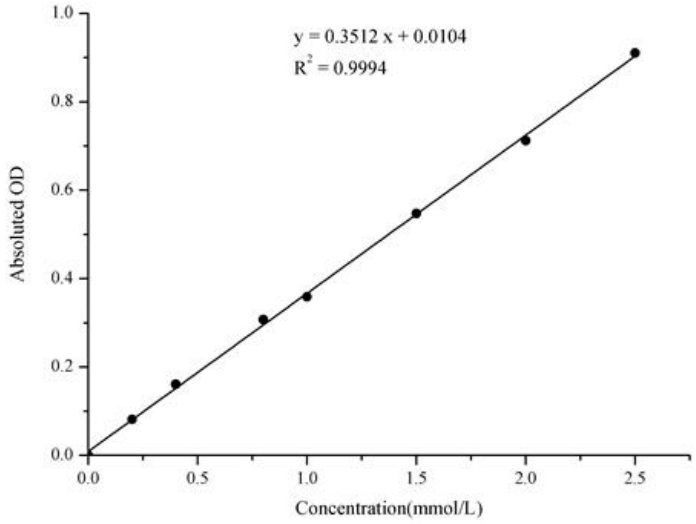
Parameters	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.35	0.88	1.7
Observed Conc. (mmol/L)	0.4	0.9	1.8
Recovery rate (%)	101	104	104

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.

Standard curve

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



Standard curve data

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
Absolute OD	0	0.081	0.161	0.307	0.359	0.547	0.713	0.911

13. Appendix II Example Analysis

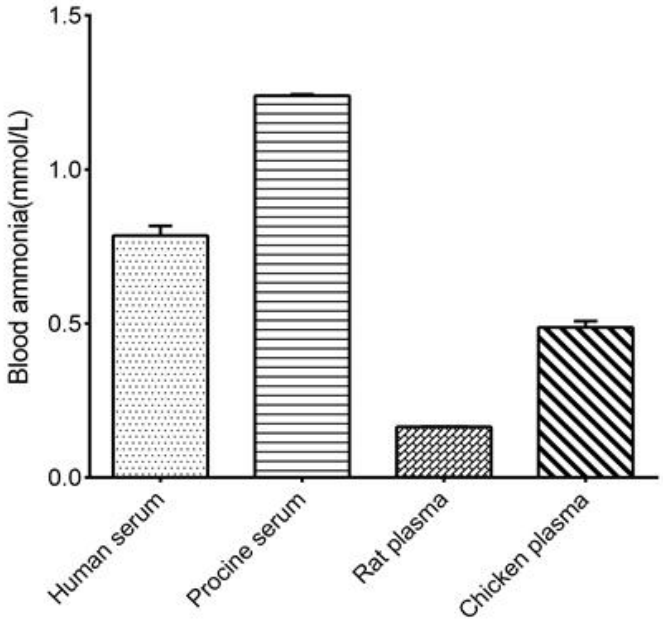
Example analysis:

Take 100 µL of human serum and perform the assay according to the operating steps. The results are as follows:

Standard curve: y = 0.3198x – 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:

Blood ammonia (mmol/L) = (0.314 - 0.050 + 0.0124) ÷ 0.3198 = 0.86 mmol/L

Detection of human serum, porcine serum, rat plasma, and chicken plasma according to the protocol showed the following results:



14. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. The experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality

of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

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Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.