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

Catalog No：E-BC-K777-M

Specification：96T (40 samples)

Measuring instrument：Microplate reader(330-350 nm)

Detection range：0.01-2.00 mmol/L

Elabscience® 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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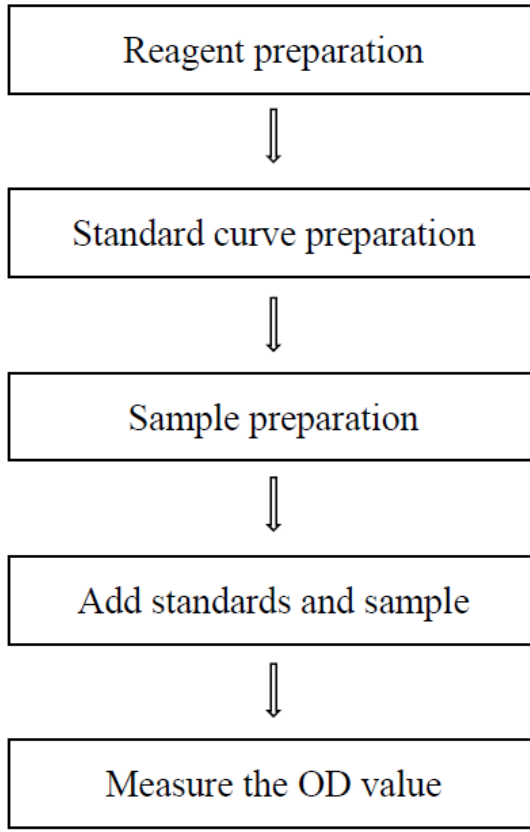
Assay summary



Intended use

This kit can be used to measure ammonia content in serum (plasma), urine, animal (plant) tissue and cell samples.

Detection principle

Ammonia is an important source of nitrogen for living systems and is necessary for the synthesis of amino acids. Ammonia is a metabolite produced by the deamination of amino acids. It plays an important role in both normal and abnormal animal physiology. In severe liver diseases, ammonia can not be removed from the circulation, resulting in increased blood ammonia, which can lead to hepatic encephalopathy (hepatic coma). In clinical practice, blood ammonia measurement is an important experimental diagnosis and monitoring index of hepatic encephalopathy.

The detection principle of this kit: enzyme-catalyzed ammonia reaction consumes NADH, causing its absorbance to decline at 340 nm. By measuring the rate of absorbance decline at 340 nm, the ammonia content can be measured.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	40 mL ×2 vials	-20℃, 12 months, shading light
Reagent 2	Substrate	1.5 mL ×1 vial	-20℃, 12 months, shading light
Reagent 3	Accelerant	Powder ×2 vials	-20℃, 12 months, shading light
Reagent 4	Catalyst	1.5 mL ×1 vial	-20℃, 12 months, shading light
Reagent 5	10 mmol/L Standard Solution	1 mL ×1 vial	-20℃, 12 months, shading light
	UV-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 (330-350 nm, optimum wavelength: 340 nm), Incubator (37 ℃)

Reagents:

Normal saline (0.9% NaCl)

Consumptive material:

10 kDa MWCO Spin Filter

Reagent preparation

① Equilibrate all the reagents to 25 ℃ before use.

② The preparation of accelerant working solution:
Dissolve one vial of accelerant with 750 μL of buffer solution, mix well to dissolve. Keep it on ice during use protected from light. Store at -20 ℃ for 5 days protected from light.

③ The preparation of measuring working solution:
Before testing, please prepare sufficient measuring working solution according to the test wells. For example, prepare 270 μL of measuring working solution (mix well 200 μL of buffer solution, 20 μL of substrate, 10 μL of accelerant working solution and 40 μL of catalyst). Keep it on ice during use protected from light and used up within the same day.

④ The preparation of control working solution:
Before testing, please prepare sufficient control working solution according to the test wells. For example, prepare 270 μL of control working solution (mix

well 240 μL of buffer solution, 20 μL of substrate and 10 μL of accelerant working solution). Keep it on ice during use protected from light and used up within the same day.

⑤ The preparation of 2 mmol/L standard solution:

Before testing, please prepare sufficient 2 mmol/L standard solution. For example, prepare 1000 μL of 2 mmol/L standard solution (mix well 200 μL of 10 mmol/L standard solution and 800 μL of buffer solution). Keep it on ice during use protected from light. Store at -20℃ for 2 days.

⑥ The preparation of standard curve:

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

Dilute 2 mmol/L standard solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 0.2, 0.4, 0.8, 1.0, 1.6, 1.8, 2.0 μmol/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**	**1.6**	**1.8**	**2.0**
2 mmol/L Standard (μL)	0	20	40	80	100	160	180	200
Buffer solution (μL)	200	180	160	120	100	40	20	0

Sample preparation

① Sample preparation

Serum (plasma) and urine sample: Add 200-400 μL of liquid sample into 10 kDa MWCO Spin Filter and centrifuge at 12000×g for 25 min at 4℃, collect the filtrate. Keep it on ice for detection and store at -20℃ for 3 days.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 100 mg).

② Wash tissue in cold normal saline (0.9% NaCl).

③ Homogenize 100 mg tissue in 900 μL buffer solution with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material.

⑤ Collect supernatant and add it to 10 kDa MWCO Spin Filter. Centrifuge at 12000×g for 25 min at 4℃.

⑥ Collect the filtrate and preserve it on ice for detection. Store at -20℃ for 3 days.

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Homogenize 1×10^6 cells in 200 μL buffer solution with a dounce homogenizer at 4℃.

③ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material.

④ Collect supernatant and add it to 10 kDa MWCO Spin Filter. Centrifuge at 12000×g for 25 min at 4℃.

⑤ Collect the filtrate and preserve it on ice for detection. Store at -20℃ for 3 days.

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Mouse serum (plasma)	1
Rat serum	1
Human serum	1
Human urine	5-10
10% Mouse liver tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse brain tissue homogenate	1
10% Broccoli tissue homogenate	1
10% Bean sprout tissue homogenate	1
1×10^6 Molt-4 cells	1
1×10^6 Jurkat cells	1
1×10^6 293T cells	1

Note: The diluent is buffer solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

If the sample is not detected or the measured value is low, the incubation time can be appropriately extended to determine again.

Operating steps

① Standard well: Add 20 μL of different concentrations solution to standard well.

Sample well: Add 20 μL of sample to sample well.

Control well: Add 20 μL of sample to control well.

② Add 140 μL of measuring working solution to standard and sample wells. Add 140 μL of control working solution to control wells.

③ Mix fully with microplate reader for 5 s. Measure the OD value of each well at 340 nm with microplate reader, as A_1. Incubated at 25℃ for 15 min, measure the OD value of each well at 340 nm with microplate reader, as A_2. $\Delta A = A_1 - A_2$.

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:

1. Serum (plasma) and urine sample:

$$\text{Ammonia content (mmol/L)} = (\Delta A_{340} - b) \div a \times f$$

2. Tissue sample:

$$\text{Ammonia content (μmol/g wet weight)} = (\Delta A_{340} - b) \div a \div m \times v \times f$$

3. Cell sample:

$$\text{Ammonia content (μmol/10^6)} = (\Delta A_{340} - b) \div a \div n \times v \times f$$

[Note]

ΔA_{340}: $\Delta A_{340} = \Delta A_{sample} - \Delta A_{control}$, $\Delta A = A_1 - A_2$.

m: The wet weight of sample, g.

n: The number of cell samples, 10^6.

v: The volume of buffer solution in the preparation step, mL.

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)	0.50	1.00	1.50
%CV	0.9	1.2	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)	0.50	1.00	1.50
%CV	7.2	7.8	9.6

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 108.3%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.50	1.00	1.50
Observed Conc. (mmol/L)	0.54	1.08	1.65
recovery rate(%)	107	108	110

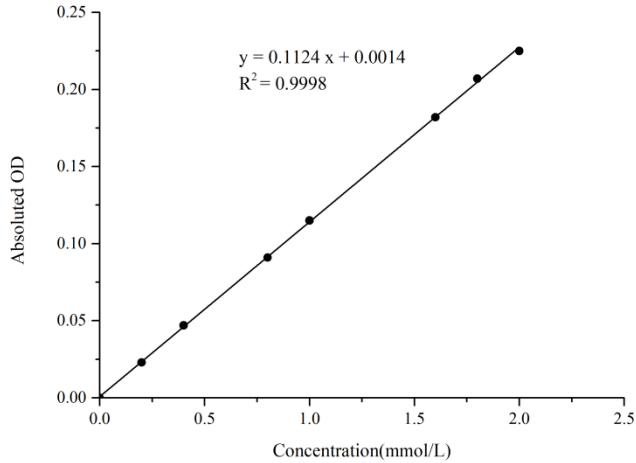
Sensitivity

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**	**1.6**	**1.8**	**2**
A_1	1.522	1.517	1.503	1.505	1.498	1.471	1.475	1.463
	1.504	1.510	1.498	1.490	1.462	1.481	1.459	1.452
A_2	1.511	1.482	1.445	1.405	1.371	1.278	1.255	1.225
	1.492	1.475	1.439	1.385	1.337	1.288	1.242	1.217
ΔA	0.011	0.035	0.058	0.100	0.127	0.193	0.220	0.238
	0.012	0.035	0.059	0.105	0.125	0.193	0.217	0.235
Average ΔA	0.012	0.035	0.058	0.103	0.126	0.193	0.219	0.237
Absoluted ΔA	0	0.023	0.047	0.091	0.115	0.182	0.207	0.225



Appendix Π Example Analysis

Example analysis:

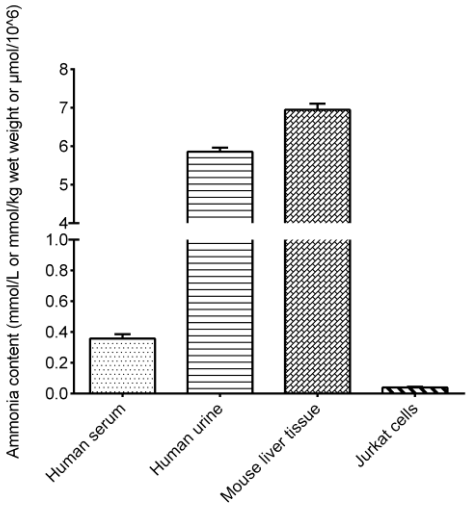
Take 20 μL of 10% mouse liver tissue homogenate and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.1124 x + 0.0014. The A_1 of the sample well is 1.206, The A_2 of the sample well is 1.102, ΔA_{sample} = 1.206 - 1.102 = 0.104, The A_1 of the control well is 1.370, The A_2 of the control well is 1.358, $\Delta A_{control}$ = 1.370 – 1.358 = 0.012, $\Delta A_{340} = \Delta A_{sample} - \Delta A_{control}$ = 0.104 – 0.012 = 0.092, and the calculation result is:

$$\text{Ammonia content (μmol/g wet weight)} = (0.092 - 0.0014) \div 0.1124 \div 0.1 \times 0.9$$

$$= 7.254 \text{ μmol/g wet tissue}$$

Detect human serum, human urine (dilute for 20 times), 10% mouse liver tissue homogenate (dilute for 2 times) and 1 ×10^6 Jurkat cells, 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.