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

Catalog No：E-BC-K183-M

Specification：96T(40 samples)/500Assays(242 samples)

Measuring instrument：Microplate reader（565 -595 nm）

Detection range：0.28-35 mmol/L

Elabscience® Urea (BUN) Colorimetric Assay Kit

(Urease Method)

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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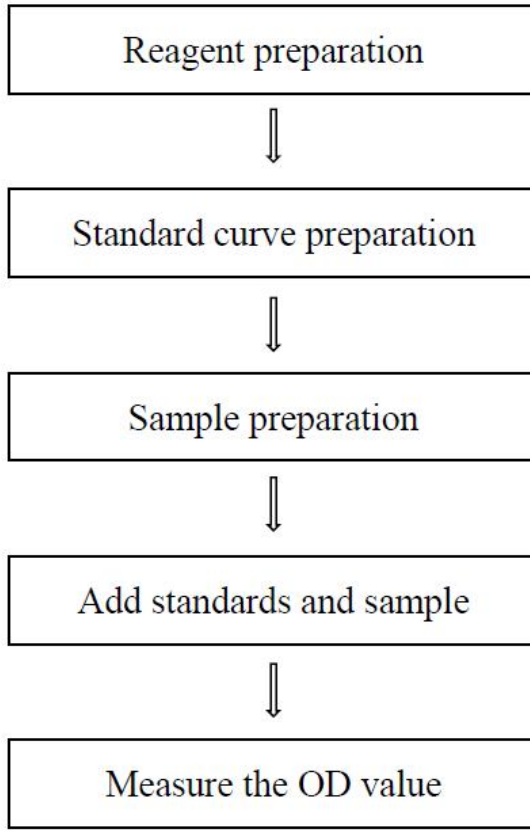
Assay summary



Intended use

This kit can be used to measure urea content in serum, plasma, urine, saliva, milk samples.

Detection principle

Urea can be decomposed into ammonia ion and carbon dioxide by urease. Ammonia ion can react with amphyl and form a green substance in alkaline medium, and the production of the green substance is proportional to the urea content which can be calculated with the colorimetric assay at 580 nm.

Kit components & storage

Item	Component	Size 1(96 T)	Size 2(500 Assays)	Storage
Reagent 1	100 mmol/L Urea Standard	2 mL × 1 vial	10 mL × 1 vial	2-8°C, 12 months
Reagent 2	Enzyme Stock Solution	0.05 mL × 1 vial	0.25 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 3	Enzyme Diluent	15 mL × 1 vial	40 mL × 2 vial	2-8°C, 12 months
Reagent 4	Chromogenic Agent	15 mL × 1 vial	40 mL × 2 vial	2-8°C, 12 months, shading light
Reagent 5	Alkaline NaClO	15 mL × 1 vial	40 mL × 2 vial	2-8°C, 12 months, shading light
	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 (565-595 nm, optimum wavelength: 580 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer

Reagents:

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of enzyme working solution:

Before testing, please prepare sufficient enzyme working solution according to the test wells. For example, prepare 1505 μL of enzyme working solution (mix well 5 μL of enzyme stock solution and 1500 μL of enzyme diluent). The enzyme working solution should be prepared on spot.

③ The preparation of standard curve：

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

Dilute 100 mmol/L urea standard with deionized water to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 15, 20, 25, 30, 35 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**5**	**10**	**15**	**20**	**25**	**30**	**35**
100 mmol/L standard (μL)	0	10	20	30	40	50	60	70
Deionized water (μL)	200	190	180	170	160	150	140	130

Sample preparation

① Sample preparation:

Serum (plasma) samples: detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for a month.

Urine: Collect fresh urine and centrifuge at 10000×g for 10 min at 4°C. Take the supernatant to preserve it on ice for detection. If not detected on the same day, the urine can be stored at -80°C for a month.

Saliva: Gargle with clear water, collect the saliva 30 min later, centrifuge at 10000×g for 10 min at 4°C. Take the supernatant and preserve it on ice for detection. If not detected on the same day, the saliva can be stored at -80°C for a month.

Milk: Collect fresh milk, centrifuge at 10000×g for 10 min at 4°C, remove the upper layer of milky white, take the middle layer supernatant and preserve it on ice for detection. If not detected on the same day, the milk can be stored at -80°C for a month.

② Dilution of sample

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

Sample type	Dilution factor
Human serum	1
Rat plasma	1
Human saliva	1
Human urine	50-70

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M，pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① Properly dilute the sample if the color is too dark, and multiply by dilution factor when calculating the result.

② It is recommended to use disposable plastic tubes to avoid contamination.

③ Prepare fresh enzyme working solution for needed amount before use. The enzyme working solution cannot be store for a long time.

④ The adhesion of enzyme stock solution is strong. It should be slowly absorbed when absorbing with pipette.

⑤ The incubation time must be 10 min accurately after adding enzyme working solution.

Operating steps

① Standard wells: Add 4 μL of standard solution with different concentrations to the corresponding wells.

Sample wells: Add 4 μL of sample to the corresponding wells.

Control wells: Add 4 μL of sample to the corresponding wells.

② Add 50 μL of enzyme working solution to standard wells and sample wells, add 50 μL of enzyme diluent to control wells, mix fully with microplate reader for 10 s, then react at 37°C for 10 min accurately.

③ Add 125 μL of chromogenic agent and 125 μL of alkaline NaClO to each well, mix fully with microplate reader for 10 s, react at 37°C for 10 min accurately.

④ Measure the OD value of each well at 580 nm with microplate reader.

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:

Serum (plasma) and other liquid samples:

$$\text{Urea content (mmol/L)} = (\Delta A_{580} - b) \div a \times f$$

[Note]

f: Dilution factor of sample before test.

ΔA_{580}: $OD_{Sample} - OD_{Control}$.

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)	1.50	12.70	26.00
%CV	3.3	2.7	2.4

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)	1.50	12.70	26.00
%CV	4.1	4.5	4.3

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)	8.5	18	29.5
Observed Conc. (mmol/L)	8.8	19.3	30.1
Recovery rate (%)	103	107	102

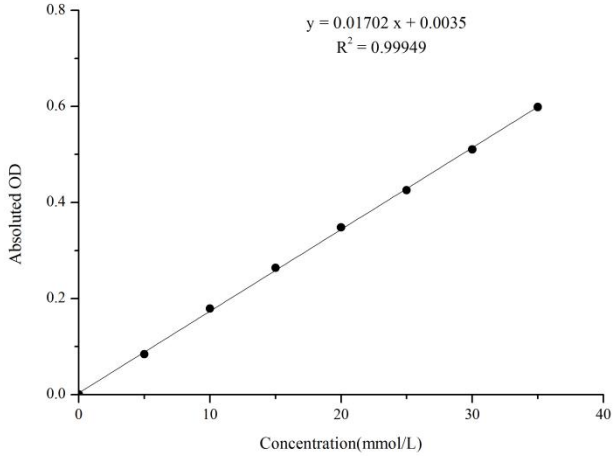
Sensitivity

The analytical sensitivity of the assay is 0.09 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	5	10	15	20	25	30	35
Average OD	0.045	0.129	0.223	0.309	0.393	0.471	0.555	0.644
Absoluted OD	0	0.084	0.179	0.264	0.348	0.426	0.510	0.599



Appendix Π Example Analysis

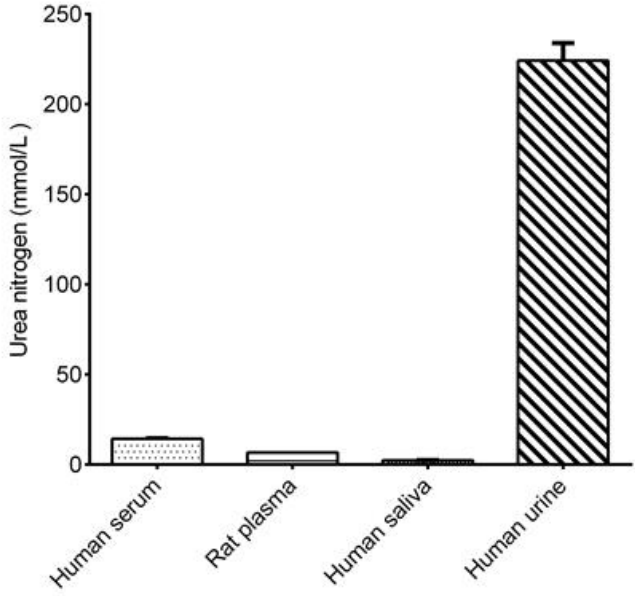
Example analysis:

Take 4 μL of rat plasma sample, carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.01702 x + 0.0035, the average OD value of the sample well is 0.249, the average OD value of the control well is 0.112, and the calculation result is:

$$\text{Urea content (mmol/L)} = (0.249 - 0.112 - 0.0035) \div 0.01702 = 7.84 \text{ mmol/L}$$

Detect human serum, rat plasma, human saliva, human urine (dilute for 50 times) 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.