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

Catalog No：E-BC-K329-S

Specification：50 Assays(48 samples)/ 100 Assays(96 samples)

Measuring instrument：Spectrophotometry（520 nm）

Detection range：0.12-15 mmol/L

Elabscience® Urea (BUN) Colorimetric Assay Kit

(Diacetyl Oxime Method)

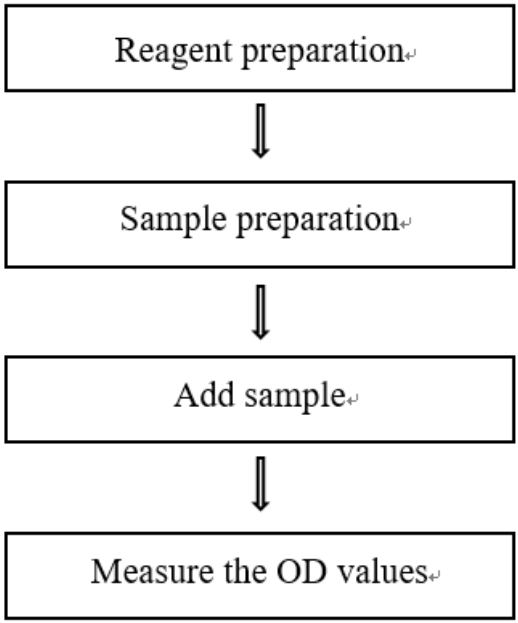
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

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.

Table of contents

Assay summary

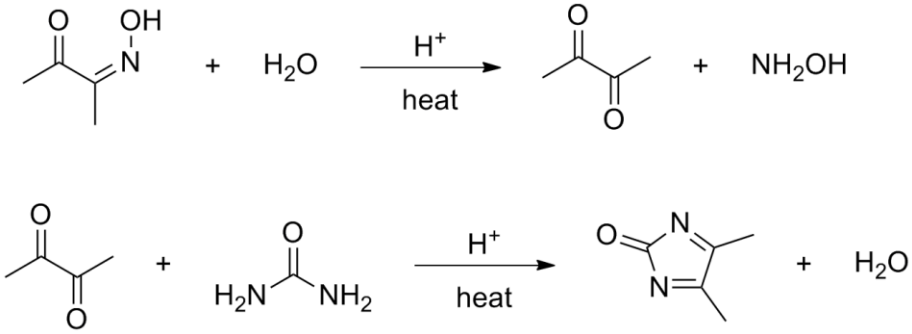


Intended use

This kit can be used to measure the urea content in serum, plasma, urine and other samples.

Detection principle

In strong acidic and heating condition, urea can react with diacetyl to form red diazine compound. The depth of color is proportional to the content of urea. Because the instability of the diacetyl, the diacetyl oxime usually react with the strong acid firstly in the reaction system to generate diacetyl, then react with urea to generate the red diazine compound. The reaction equation is as follows.



Kit components & storage

Item	Component	Size 1 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Oxime Solution	60 mL ×1 vial	60 mL ×2 vials	2-8 ℃, 12 month shading light
Reagent 2	Acid Solution	20 mL ×1 vial	40 mL ×1 vial	2-8 ℃, 12 month shading light
Reagent 3	10 mmol/L Urea Standard	1 mL ×1 vial	1 mL ×1 vial	2-8 ℃, 12 months

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:

Spectrophotometer (520 nm), Vortex mixer, Micropipettor, Incubator， Water bath

Reagents:

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

Reagent preparation

① The preparation of acid working solution：

For each tube, prepare 1050 μL of acid working solution (mix well 350 μL of acid solution and 700 μL of double distilled water). The acid working solution should be prepared on spot.

② It is recommended to aliquot the 10 mmol/L urea standard and store at 4 ℃.

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80 ℃ for a month.

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

Saliva: Gargle with clear water, collect the saliva 30 min later, centrifuge at 10000 g for 10 min at 4 ℃. Take the supernatant and preserve it on ice for detection.

② Dilution of sample

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

Sample type	Dilution factor
Human urine	45-60
Human plasma	1
Human serum	1
Human saliva	1
Mouse serum	1
Rat urine	45-60
Rabbit plasma	1

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

Operating steps

① Open the water bath in advance and set the temperature to 100 ℃.

② Blank tube: add 20 μL of double distilled water into 5 mL glass tube.
Standard tube: add 20 μL of 10 mmol/L urea standard into 5 mL glass tube.
Sample tube: add 20 μL of sample into 5 mL glass tube.

③ Add 1000 μL of oxime solution and 1000 μL of acid working solution into each tube. Tight the tubes with preservative film and mix fully with vortex mixer. Incubate the tubes in boiling water for 15 min. Cool the tubes with running water.

④ Set to zero with double-distilled water and measure the OD value of each tube with 1 cm optical path cuvette at 520 nm.

Calculation

The sample:

$$\text{Urea content (mmol/L)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f$$

[Note]

ΔA_1: $OD_{Sample} - OD_{Blank}$.

ΔA_2: $OD_{Standard} - OD_{Blank}$

c: Concentration of urea standard .

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)	0.84	5.30	10.80
%CV	5.3	4.7	4.7

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.84	5.30	10.80
%CV	9.4	10.2	10.1

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 101%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (mmol/L)	2.6	8.4	12.5
Observed Conc. (mmol/L)	2.6	8.7	12.6
Recovery rate (%)	99	103	101

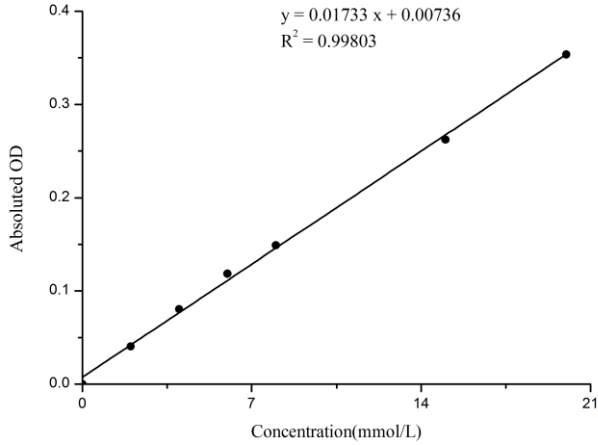
Sensitivity

The analytical sensitivity of the assay is 0.12 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**	**2**	**4**	**6**	**8**	**15**	**20**
Average OD	0.005	0.046	0.086	0.124	0.154	0.268	0.359
Absoluted OD	0	0.041	0.081	0.119	0.149	0.263	0.354



Appendix II Example Analysis

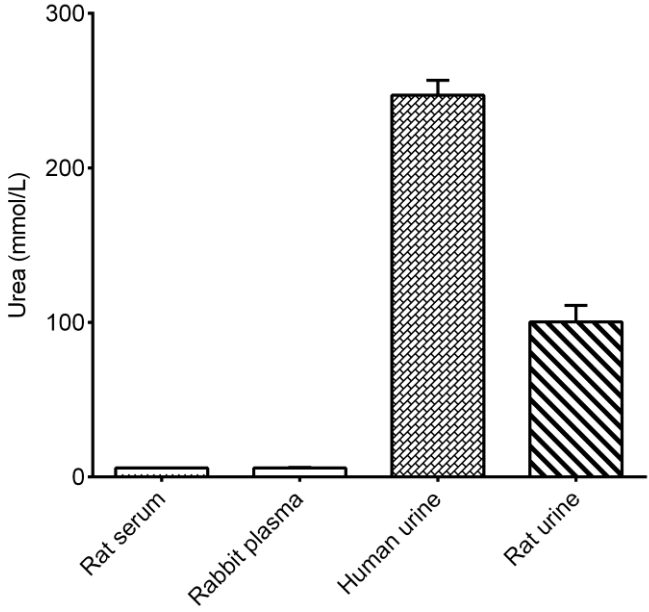
Example analysis:

Take 0.02 mL of rat serum, and carry the assay according to the operation steps. The results are as follows:

the average OD value of the blank is 0.004, the average OD value of the standard is 0.343, the average OD value of the sample is 0.199, and the calculation result is:

$$\text{Urea content (mmol/L)} = \frac{0.199 - 0.004}{0.343 - 0.004} \times 10 \times 1 = 5.75 \text{ mmol/L}$$

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