Urea (BUN) Colorimetric Assay Kit
(Urease Method)

Catalog No: E-BC-K183-M

Method: Colorimetric method

Specification: 96T (Can detect 40 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 0.09 mmol/L

Detection range: 0.28-35 mmol/L

- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

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

▲Background

Urea is the major final-product of protein metabolism in the body, which constitutes the clear majority of non-protein nitrogen in blood. Blood urea nitrogen come from the liver, which excreted with urine through kidney. Renal function failure, nephritis, urinary tract obstruction and so on can make the content of blood urea increased. Urea is the largest nitrogen circulating sediment except the nitrogen in circulating protein, and it is also the main carrier of removing harmful ammonia in the body.

▲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	Specification	Storage
Reagent 1	100 mmol/L Urea Standard	2 mL × 1 vial	2-8℃ , 6 months
Reagent 2	Enzyme Stock Solution	0.05 mL × 1 vial	2-8℃ , 6 months, shading light
Reagent 3	Enzyme Diluent	15 mL × 1 vial	2-8℃ , 6 months
Reagent 4	Chromogenic Agent	15 mL × 1 vial	2-8℃ , 6 months, shading light
Reagent 5	Alkaline NaClO	15 mL × 1 vial	2-8℃ , 6 months, shading light
	Microplate	96 wells	
	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.			

▲Materials prepared by users

Instruments

Microplate reader (565-595 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL, 5 mL)

Reagents:

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

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

▲Precautions

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

▲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.

Pre-assay preparation

▲Reagent preparation

Preparation of enzyme working solution

Prepare fresh enzyme working solution according to the ratio of reagent 2: reagent 3=1:300 before use.

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

Sample requirements

Heparin ammonium should not be used as an anticoagulant.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.28-35 mmol/L).

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).

Assay protocol	
Ambient temperature	25-30℃
Optimum detection wavelength	580nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'
B	B	B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'
C	C	C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'
D	D	D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
E	E	E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
F	F	F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
G	G	G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
H	H	H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'

[Note]: A-H, standard wells; S1-S40, sample wells; S1'-S40', control wells.

▲Operating steps

The preparation of standard curve

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.

The measurement of samples

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 reagent 3 to control wells, mix fully with microplate reader for 10 s, then react at 37℃ for 10 min accurately.
3) Add 125 μL of reagent 4 and 125 μL of reagent 5 to each well, mix fully with microplate reader for 10 s, react at 37℃ for 10 min accurately.
4) Measure the OD value of each well at 580 nm with microplate reader.

▲Operation table

	Standard well	Sample well	Control well
Standard solution with different concentrations (μL)	4		
Sample (μL)		4	4
Enzyme working solution (μL)	50	50	
Reagent 3 (μL)			50
Mix fully with microplate reader for 10 s, react at 37℃ for 10 min accurately.			
Reagent 4 (μL)	125	125	125
Reagent 5 (μL)	125	125	125
Mix fully with microplate reader for 10 s and react at 37℃ for 10 min accurately. Measure the OD values of each well at 580 nm with microplate reader.			

▲Calculation

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.

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

Note:

y: $OD_{Standard}$ – OD_{Blank}.(OD_{Blank} is the OD value when the standard concentration is 0).

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

f: Dilution factor of sample before test.

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

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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Detection range	0.28-35 mmol/L	Average intra-assay CV (%)	2.8
Sensitivity	0.09 mmol/L	Average inter-assay CV (%)	4.3
Average recovery rate (%)	104		

▲Example analysis

Take 4 μL of rat plasma sample, carry the assay according to the operation table. 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 blank well is 0.112, and the calculation result is:

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

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80℃ for a month.

▲ Plasma

Take fresh blood into the tube which has anticoagulant, centrifuge at 700-1000 g for 10 min at 4℃ . Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the 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. If not detected on the same day, the urine can be stored at -80℃ for a month.

▲ Milk

Collect fresh milk, centrifuge at 10000 g for 10 min at 4℃ , 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 urine can be stored at -80℃ for a month.

▲ Notes for sample

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
2. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.