PRODUCT INFORMATION & MANUAL

Urea Assay Kit (Colorimetric)

NBP3-24545

For research use only.
Not for diagnostic or therapeutic procedures.

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Urea (BUN) Colorimetric Assay Kit
(Urease Method)

Catalog No: NBP3-24545

Method: Colorimetric method

Specification: 100 Assays (Can detect 48 samples without duplication)

Measuring instrument: Spectrophotometer

Sensitivity: 0.114 mmol/L

Detection range: 0.114-30 mmol/L

Average intra-assay CV (%): 4.6

Average inter-assay CV (%): 4.7

Average recovery rate (%): 102

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ 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 (BUN) content in animal serum, plasma, urine and 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.

▲Detection principle

Urea can be decomposed into ammonia ion and carbon dioxide by urease. Ammonia ion can react with phenol chromogenic agent and form a blue substance in alkaline medium, and the production of the blue 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℃ , 12 months
Reagent 2	Enzyme Stock Solution	0.1 mL × 1 vial	2-8℃ , 12 months, shading light
Reagent 3	Enzyme Diluent	30 mL × 1 vial	2-8℃ , 12 months
Reagent 4	Chromogenic Agent	60 mL × 2 vials	2-8℃ , 12 months, shading light
Reagent 5	Alkaline NaClO	60 mL × 2 vials	2-8℃ , 12 months, shading light
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

Spectrophotometer (580 nm), Micropipettor, Vortex mixer, Incubator

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. Therefore, it is better to make batch operation if there are many samples to be detected. The number of operation in a batch should be less than 20.

Pre-assay preparation

▲ Reagent preparation

1. Preparation of 10 mmol/L urea standard working solution:
   Dilute the 100 mmol/L urea standard with double distilled water at 1: 9. Store at 2-8℃ for 3 days.

2. 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.

▲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 (0.114-30 mmol/L).

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

Sample type	Dilution factor
Rabbit plasma	1
Rat serum	1
Rat plasma	1
Human serum	1
Human saliva	1
Human milk	1
Human urine	30-60
Mouse urine	30-60

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

Assay protocol

▲Detailed operating steps

1. Blank tube: Add 0.02 mL of double distilled water to the 5 mL EP tube.

   Standard tube: Add 0.02 mL of 10 mmol/L urea standard working solution to the 5 mL EP tube.

   Sample tube: Add 0.02 mL of sample to the 5 mL EP tube.

   Control tube: Add 0.02 mL of sample to the 5 mL EP tube.

2. Add 0.25 mL of enzyme working solution to blank tube, standard tube and sample tube of step 1, add 0.25 mL of reagent 3 to control tube, mix fully with vortex mixer, incubate at 37℃ for 10 min.
3. Add 1 mL of reagent 4 and 1 mL of reagent 5, mix fully, incubate at 37℃ for 10 min.
4. Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube with 1 cm optical path cuvette at 580 nm.

▲Summary operation table

	Blank tube	Standard tube	Sample tube	Control tube
Double distilled water (mL)	0.02			
10 mmol/L urea standard working solution (mL)		0.02		
Sample (mL)			0.02	0.22
Enzyme working solution (mL)	0.25	0.25	0.25	
Reagent 3 (mL)				0.25
Mix fully, incubate in 37℃ for 10 min accurately.				
Reagent 4 (mL)	1	1	1	1
Reagent 5 (mL)	1	1	1	1
Mix fully and incubate in 37℃ for 10 min. Set to zero with double distilled water and measure the OD values at 580 nm.				

▲Calculation

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

Note:

ΔA_1: $OD_{Sample} - OD_{Control}$

ΔA_2: $OD_{Standard} - OD_{Blank}$

c: Concentration of standard (10 mmol/L urea nitrogen=280.1 mg/L)

f: Dilution factor of sample before test.

Appendix I Data

▲Example analysis

Dilute human urine with 0.9% NaCl at the ratio of 1:49, take 0.02 mL of diluted human urine, and carry the assay according to the operation table.

The results are as follows:

The average OD value of the sample is 0.134, the average OD value of the blank is 0.010, the average OD value of the standard is 0.175, the average OD value of the control is 0.017, and the calculation result is:

Urea nitrogen content (mmol/L) = (0.134 - 0.017)÷ (0.175 - 0.010)× 10 × 50 = 354.55 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 (Do not use ammonium heparin as an 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 15 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 serum 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 serum can be stored at -80℃ for a month.

Appendix III References

1. Walker V. Ammonia toxicity and its prevention in inherited defects of the urea cycle[J]. Diabetes Obesity & Metabolism, 2009, 11(9): 823-835.
2. Dimski D S. Ammonia metabolism and the urea cycle: function and clinical implications[J]. Journal of Veterinary Internal Medicine,1994, 8(2): 73-78.
3. Weiner I D, Mitch W E, Sands J M. Urea and Ammonia Metabolism and the Control of Renal Nitrogen Excretion[J]. Clin J Am Soc Nephrol, 2014, 10(8): 1444-1458.