PRODUCT INFORMATION & MANUAL

Urea Assay Kit (Colorimetric)

NBP3-24543

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

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Urea Assay Kit (Colorimetric)

Catalog No: NBP3-24543

Method: Colorimetric method

Specification: 100 Assays (Can detect 96 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 0.12 mmol/L

Detection range: 0.12-15 mmol/L

Average intra-assay CV (%): 4.9

Average inter-assay CV (%): 9.9

Average recovery rate (%): 101

- 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

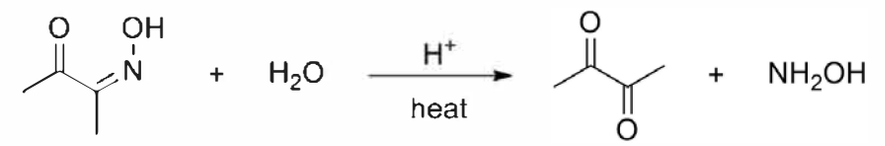
This kit can be used to measure the urea content in serum, plasma, urine and saliva samples.

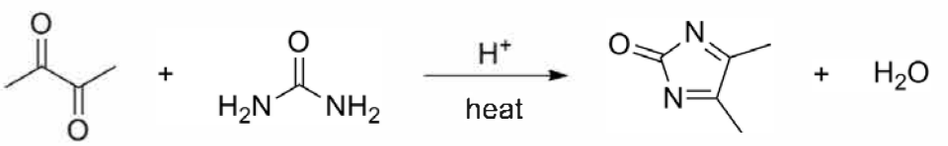
▲Background

Urea is the final decomposition product of nitrogen metabolism in animals. Biological urea is produced in the liver and excreted by the kidneys. Urea is the largest nitrogen circulating sediment in addition to circulating protein nitrogen, and is also the main carrier to remove harmful ammonia in the body.

▲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	Specification	Storage
Reagent 1	Oxime Solution	60 mL × 2 vials	2-8℃, 12 months, shading light
Reagent 2	Acid Solution	40 mL × 1 vial	2-8℃, 12 months, shading light
Reagent 3	10 mmol/L Urea Standard	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.			

▲Materials prepared by users

Instruments

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

Reagents:

Double distilled water, Normal saline (0.9%NaCl), PBS (0.01M, 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. Seal the test tube mouth with the preservative film and make a hole in the preservative film before incubating in the boiling water bath.
2. When incubate in boiling water bath, the liquid level of water bath must be higher than that of reagent in glass tube.
3. The time of incubation in boiling water bath must be accurately and cool the tubes with running water after incubation.

Pre-assay preparation

▲Reagent preparation

1. Preparation of reagent 2 working solution:
   Dilute the reagent 2 with double distilled water according to the ratio of 1:2 and mix fully. Prepare the fresh solution before use.
2. It is recommended to aliquot the reagent 3 and store at 4℃ .

▲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 (0.12-15 mmol/L).

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).

Assay protocol

▲Detailed 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 reagent 1 and 1000 μL of reagent 2 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.

▲Summary operation table

	Blank well	Standard well	Sample well
Double distilled water (μL)	20		
10 mmol/L urea standard (μL)		20	
Sample (μL)			20
Reagent 1 (μL)	1000	1000	1000
Reagent 2 working solution (μL)	1000	1000	1000
Mix fully. Incubate in boiling water for 15 min. Cool the tubes with running water. Measure the OD value with 1 cm optical path cuvette at 520 nm.			

▲Calculation

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

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

▲Example analysis

Take 0.02 mL of rat serum, carry the assay according to the operation table.

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:

Urea content (mmol/L) = (0.199-0.004)÷(0.343-0.004)×10×1=5.75 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 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

Saliva was collected 30 min after gargling with clear water and centrifuged at 10000 g at 4℃ for 10 min. Then take the supernatant for detection. If not detected on the same day, the saliva 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. Diabetes Obesity & Metabolism, 2009, 11(9): 823-835.
2. Dimski D S. Ammonia metabolism and the urea cycle: function and clinical implications. 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. Clin J Am Soc Nephrol, 2014, 10(8): 1444-1458.