Technical Manual

Urea (BUN) Colorimetric Assay Kit (Urease Method)

- Catalogue Code: MAES0136
- Size: 96T
- Research Use Only

1. Key Features and Sample Types:

Detection method:

Colorimetric method

Specification:

96T

Range:

0.28-35 mmol/L

Sensitivity:

0.09 mmol/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. 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.

3. Intended Use:

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

4. 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.

5. Kit Components & Storage:

Item	Specification	Storage
Urea Standard (100 mmol/L)	2 mL × 1 vial	2-8°C, 6 months
Enzyme Stock Solution	0.05 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Enzyme Diluent	15 mL × 1 vial	2-8°C, 6 months
Chromogenic Agent	15 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Alkaline NaClO	15 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (565-595 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)

6. Assay Notes:

1. It is recommended to use disposable plastic tubes to prevent contamination.

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

7. Reagent Preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **enzyme working solution:** Prepare fresh enzyme working solution according to the ratio of enzyme stock solution: enzyme diluent = 1:300 before use.

8. Sample Preparation:

Sample requirements: Heparin ammonium should not be used as an anticoagulant.

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

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

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

4. 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 on ice before detection. If not detected on the same day, the urine can be stored at -80°C for a month.

5. 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 on ice before detection. If not detected on the same day, the urine can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. 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
Human saliva	1
Rat plasma	1
Human saliva	1

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

9. Assay Protocol:

Ambient Temperature: 25-30°C

Optimum detection wavelength: 580 nm

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.

10. Operation 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 well:** Add 4 μL of **standard solution with different concentrations** to the corresponding wells.
   Sample well: Add 4 μL of **sample** to the corresponding wells.
   Control well: 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 10s, 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 10s, react at 37°C for 10 min accurately.

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

Operation Table

	Blank well	Standard well	Sample well
Standard solution with different concentrations (μL)	4		
Sample (μL)		4	4
Enzyme working solution (μL)	50	50	
Enzyme diluent (μL)			50
Mix fully with microplate reader for 10s, react at 37°C for 10 min accurately.			
Chromogenic agent (μL)	125	125	125
Alkaline NaClO (μL)	125	125	125
Mix fully with microplate reader for 10s and react at 37°C for 10 min accurately. Measure the OD values of each well at 580 nm with microplate reader.			

11. Calculations:

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.

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

y: $OD_{Standard} - OD_{Blank}$.

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}: $OD_{Sample} - OD_{Control}$

12. Performance Characteristics:

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

Analysis

Take 4 μL of rat plasma, 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:

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

$$= 7.84 \text{ mmol/L}$$

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching 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.

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

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