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Datasheet

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Urea Colorimetric Assay Kit

Cat# AS1001

Store at 2-8˚C for 6 month

INTRODUCTION

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

DETECTION SIGNIFICANCE

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.

SPECIFICATION

Method: Colorimetric method

Sensitivity: 0.09 mmol/L.

Detection Range: 0.28-35 mmol/L

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

Measuring instrument: Microplate reader

PRINCIPLE of KIT

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.

CONTENTS and STORAGE

An unopened kit can be stored at 2-8℃ for 1 month. If the kit is not used within 1 month, store the items separately according to the following conditions once the kit is received

	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	6 mL × 1 vial	2-8℃, 6 months
Preparation of enzyme working solution: Prepare fresh enzyme working solution according to the ratio of Enzyme stock solution: Enzyme diluent=1:300 before use.			

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

Experimental instruments : *Test tube, Micropipettor, Vortex mixer, Water bath/Incubator, Centrifuge, Spectrophotometer (580 nm)*

PREPARATION OF SAMPLE

1. **Plasma (serum):** Detect directly. Samples can be store at room temperature for 24 hours or at 4℃ for 7 days. Use oxalate, heparin or EDTA as the plasma anticoagulant.
2. **Urine:** Collect the fresh urine and centrifuge the sample at 10000 g for 10 min at 4℃. Take the supernatant and dilute the urine with normal saline at a ratio of 1:19~1:49. Increase the dilution multiple of sample if the result exceeds the linear range.
3. **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.
4. **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

OPERATION STEPS

1. Preparation of standard: Dilute 100 mmol/L Urea Standard with double-distilled water to a serial concentration. The recommended dilution gradient is as follows: 35, 30, 25, 20, 15, 10, 5, 0 mmol/L.
2. Standard wells: Add 4 μL of standard solution with different concentrations into the wells.
3. Sample wells: Add 4 μL of Sample into the wells.
4. Control wells: Add 4 μL of Sample into the wells.
5. 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.
6. Add 125 μL of reagent 4 and 125 μL of reagent 5, mix fully with microplate reader for 10 s, react at 37 ℃ for 10 min accurately.
7. Measure the OD value of each well at 580 nm.

Note: The following operating table could be as a reference.

	Standard tube	Sample tube	Control tube
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.			

CALCULATION of RESULTS

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and xaxis 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: The absolute OD value of standard ($OD_{Standard} - OD_{Blank}$).

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}: Absolute OD ($OD_{Sample} - OD_{Control}$).

TYPICAL PARAMETERS

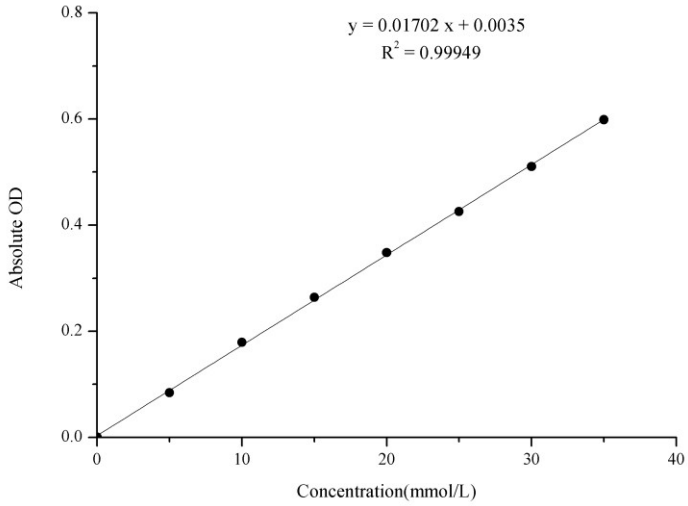
1. The sensitivity of the kit is 0.09 mmol/L.
2. The intra-assay CV is 2.8% and the inter-assay CV is 4.3%.
3. The recovery of the kit is 104%.
4. The linear range of the kit is 0.28-35 mmol/L.

NOTE

1. The kit is for scientific research 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.
5. Properly dilute the sample if the color is too dark, and multiply by dilution factor when calculating the result.
6. It is recommended to use disposable plastic tubes to avoid contamination.
7. Prepare fresh enzyme working solution for needed amount before use. The enzyme working solution cannot be store for a long time.

8. The adhesion of enzyme stock solution is strong. It should be slowly absorbed when absorbing with pipette.
9. The incubation time must be 10 min accurately after adding enzyme working solution.

Appendix: Standard curve



PRODUCT USE LIMITATION

These products are intended for research use only.