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CheKine™ Micro Urea Nitrogen Content Assay Kit

Cat #: KTB1511 Size: 96 T/96 S

	Micro Urea Nitrogen Content Assay Kit		
REF	**Cat #:** KTB1511	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal and Plant Tissues, Cells, Plasma, Serum or other Liquid samples		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Urea is the end product of nitrogenous compound degradation in living organisms, undergoing conversion to ammonia under the catalysis of urease. Blood urea nitrogen (BUN) is one of the principal indicators of renal function. In a sample, urea nitrogen reacts in a solution containing ferric chloride and phosphate, along with diacetyl monoxime and thiourea, upon heating to produce a red diazine compound. The intensity of the color of this compound is directly proportional to the concentration of urea nitrogen present. The determination of urea nitrogen levels is accomplished using the diacetyl monoxime method, which relies on colorimetric analysis of the reaction mixture to quantify urea nitrogen content in serum or urine specimens.

Materials Supplied and Storage Conditions

Kit components	Size（96 T）	Storage conditions
Reagent Ⅰ	7 mL	4℃, protected from light
Reagent Ⅱ	70 mL	4℃, protected from light
Standard	1 mL	4℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 540 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Thermostatic water bath, ice maker, centrifuge
- Deionized water
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Reagent Ⅱ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Note: Reagent Ⅰ and Reagent Ⅱ are toxic, and Reagent Ⅱ is corrosive, so it is recommended to experiment in a fume hood.

Standard: 1 mg/mL urea nitrogen standard solution. Store at 4℃.

Standard preparation: Using 1 mg/mL urea nitrogen standard solution, prepare standard curve dilution as described in the table

in a microplate or microcentrifuge tubes:

Num.	Standard Volume	Deionized Water Volume (μL)	Concentration (μg/mL)
Std.1	50 μL 1 mg/mL Standard	350	125
Std.2	200 μL of Std.1 (125 μg/mL)	200	62.5
Std.3	200 μL of Std.2 (62.5 μg/mL)	200	31.25
Std.4	200 μL of Std.3 (31.25 μg/mL)	200	15.63
Std.5	200 μL of Std.4 (15.63 μg/mL)	200	7.81
Std.6	200 μL of Std.5 (7.81 μg/mL)	200	3.9
Std.7	200 μL of Std.6 (3.9 μg/mL)	200	1.95
Blank	0	200	0

Notes: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month. When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Tissue samples: Weigh 0.1 g tissue sample, add 1 mL deionized water and homogenize on ice. Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Cells : Collect 5×10^6 cells into the centrifuge tube, wash cells with cold PBS, discard the supernatant after centrifugation; add 1 mL deionized water to ultrasonically disrupt the cells or bacteria 3 min (power 300 W, ultrasonic 3 s, interval 7 s, total time is 3 min). Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Serum (plasma) and other liquid samples: Direct determination. If the liquid is turbid, the supernatant is determined by centrifugation.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 540 nm, visible spectrophotometer was returned to zero with deionized water.
2. Operation table (The following operations are carried out in a 1.5 mL EP tube.):

Reagent	Blank Tube (μL)	Test Tube (μL)	Standard Tube (μL)
Sample supernatant	0	20	0
Standard	0	0	20
Deionized water	20	0	0
Reagent Ⅰ	50	50	50
Reagent Ⅱ	500	500	500

Mix thoroughly, then incubate in a water bath at boiling temperature for 10 min. After cooling, aspirate and transfer 200 μL to a 96-well plate. Measure the absorbance at 540 nm, recording them as A_{Blank}, A_{Test} and $A_{Standard}$, respectively. Calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: Standard curve and blank well only need to be done once or twice. Before the experiment, it is suggested that 2-3

samples with large expected differences should be selected for pre-experiment. If ΔA_{Test} is less than 0.05, increase the sample quantity appropriately. If A_{Test} is greater than 0.8, the sample supernatant can be further diluted by deionized water, and the calculation result should be multiplied by the dilution multiple, or reduce the sample size for extraction.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve:

With the concentration of the standard solution as the x-axis and the $\Delta A_{Standard}$ as the y-axis, draw the standard curve, get the standard equation, and bring the ΔA_{Test} into the equation to get the x value (μg/mL).

2. Calculation of urea nitrogen content:

(1) Calculated by protein concentration

Urea nitrogen content (μg/mg prot)=$x \times V_{Total\ Sample} \div Cpr$**=x÷Cpr**

(2) Calculated by sample fresh weight

Urea nitrogen content (μg/g fresh weight)=$x \times V_{Total\ Sample} \div W$**=x÷W**

(3) Calculated by number of cells

Urea nitrogen content (μg/10^4 cell)=$x \times V_{Total\ Sample} \div N$**=x÷N**

(4) Calculated by sample volume

Urea nitrogen content (μg/mL)**=x**

$V_{Total\ Sample}$: deionized water volume added, 1 mL; Cpr: sample protein concentration, mg/mL; W: sample weight, g, N: total number of cells, 10^4.

Typical Data

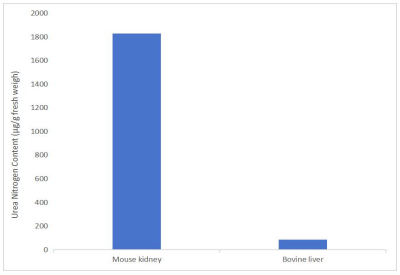

Figure 1. Determination urea nitrogen content in Mouse kidney and Bovine liver by this assay kit

Recommended Products

Catalog No.	Product Name
KTB1015	CheKine™ Micro α-Glucosidase Activity Assay Kit
KTB1121	CheKine™ Pyruvate Acid (PA) Colorimetric Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.