Urea Nitrogen/Urea Content Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/microplate reader

Cat No: JYM023CHS

Size:100T/48S, 200T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact our staff in time.

Reagent name	100T Size	200T Size	Preservation Condition
Reagent I	Powder×2	Powder×2	-20℃
Reagent II	Liquid 15 mL×1	Liquid 15 mL×1	2-8℃
Reagent III A	Liquid 3 mL×1	Liquid 3 mL×1	2-8℃
Reagent III B	Liquid 12 mL×1	Liquid 12 mL×1	2-8℃
Reagent IV	Liquid 10 mL×1	Liquid 10 mL×1	2-8℃
Standard	Powder×1	Powder×1	2-8℃

100T Solution Preparation:

1. Reagent I: Add 5 mL distilled water to per bottle before use, fully dissolved. Prepared when the solution will be used. It can be stored at 2-8 ℃ for a weeks.

200T Solution Preparation:

1. Reagent I: Add 7 mL distilled water to per bottle before use, fully dissolved. Prepared when the solution will be used. It can be stored at 2-8 ℃ for a weeks.

Preparation of other solutions:

1. Reagent III: According to the sample size, the ratio of reagent III A : reagent III B = 0.1mL : 0.4mL (total 0.5mL, about 4T) was prepared.

2. Standard: 10 mg urea. Dissolved with 4.66 mL distilled water, to make 1 mg/mL urea nitrogen standard solution (Equivalent to 2.146 mg/mL urea). It can be stored at 2-8 ℃ for 4 weeks.

3. 25μg/mL urea nitrogen standard solution (53.65 μg/mL urea standard solution): Take 25 μL 1 mg / mL urea nitrogen standard solution and add 975 μL of distilled water to prepared.

Product Description:

Urea (Urea nitrogen) is the main product of human protein metabolism. Urea constitutes the majority of non-protein nitrogen in blood. Blood urea nitrogen(urea) is one of the main indexes of renal function. This kit use indophenol blue colorimetric method to test NH_3-N product by urease hydrolysis. The concentration of indophenol is proportional to urea nitrogen(urea) concentration.

Technical Specifications:

Minimum Detection Limit:

0.00009 μg/mL(measured by urea nitrogen) or 0.000193 μg/mL(measured by urea)

Linear Range:

0.78125-100 μg/mL(measured by urea nitrogen) or 1.676-214.5 μg/mL(measured by urea)

Reagents and Equipment Required but Not Provided:

Visible spectrophotometer/microplate reader, micro glass cuvette/96 well plate, mortar/homogenizer/cell ultrasonic crusher, balance, cryogenic centrifuge, water bath, ice and distilled water.

Operation procedure:

I. Sample preparation(The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

Tissue sample: According to the ratio of mass (g): volume of distilled water 1:5-10 (it isrecommended to weigh about 0.1g and add 1mL of distilled water), homogenize on ice and then centrifuged at 4℃ 13000g for 15min, take the supernatant and placed on the ice for test.

Bacteria or cells: The ratio of bacteria/cell amount (10^4): the volume of distilled water (mL) is 500~1000:1(it is suggested to take about 5 million bacteria/cells and add 1 mL of distilled water). Bacteria/cell is split by ultrasonic (300 w, work time 3 s, interval 7 s, total time3min). Centrifuge at 13000g for 15 minutes at 4℃, take the supernatant and placed on the ice for test.

Serum (plasma) sample: Detect sample directly. If the solution is turbid, centrifuge to take the supernatant and then measure.

II. Determination procedure:

1. Preheat the spectrophotometer/microplate reader for more than 30 min, adjust the wavelength to 630 nm, set spectrophotometer zero with distilled water.
2. Operation table:

Reagent Name(μL)	Blank Tube (A_B)	Standard Tube (A_S)	Test Tube (A_T)	Control Tube (A_C)
Sample			30	30
Standard Solution		30		-
Distilled water	30			60
Reagent I	60	60	60	-
Reagent II	110	110	110	110
Mix well, place at 37℃ for 10 min.				
Reagent III	120	120	120	120
Reagent IV	90	90	90	90
Mix well, place at 37℃ for 30 min.				
Distilled water	90	90	90	90
Mix well, take 200 μL to micro glass cuvette/96 well plate, detect absorbance at 630 nm. Calculate $\Delta A_S=A_S-A_B$, $\Delta A_T=A_T-A_C$. The blank and standard tubes only need to be measured 1-2 times.				

III. Calculated by sample mass

Urea Nitrogen content (μg/g mass)= $\Delta A_T \div \Delta A_S \times C_{S1} \times V_E \div W = 25 \times \Delta A_T \div \Delta A_S \div W$

Urea content (μg/g mass)= $\Delta A_T \div \Delta A_S \times C_{S2} \times V_E \div W = 53.65 \times \Delta A_T \div \Delta A_S \div W$

1. Calculated by sample protein concentration

Urea Nitrogen content (μg/mg prot)=$\Delta A_T \div \Delta A_S \times C_{S1} \times V_E \div (Cpr \times V_E) = 25 \times \Delta A_T \div \Delta A_S \div Cpr$

Urea content (μg/mg prot)=$\Delta A_T \div \Delta A_S \times C_{S2} \times V_E \div (Cpr \times V_E) = 53.65 \times \Delta A_T \div \Delta A_S \div Cpr$

2. Calculate by the number of bacteria or cells

Urea Nitrogen content (μg/10^4 cell)= $\Delta A_T \div \Delta A_S \times C_{S1} \times V_E \div N = 25 \times \Delta A_T \div \Delta A_S \div N$

Urea content (μg/10^4 cell)= $\Delta A_T \div \Delta A_S \times C_{S2} \times V_E \div N = 53.65 \times \Delta A_T \div \Delta A_S \div N$

3. Calculated by liquid volume

Urea Nitrogen content (μg/mL)=$\Delta A_T \div \Delta A_S \times C_{S1} = 25 \times \Delta A_T \div \Delta A_S$

Urea content (μg/mL)=$\Delta A_T \div \Delta A_S \times C_{S2} = 53.65 \times \Delta A_T \div \Delta A_S$

C_{S1}: Concentration of urea standard solution, 25 μg/mL;

C_{S2}: Concentration of urea nitrogen standard solution, 53.65 μg/mL;

V_E: Extract solution volume, 1 mL;

W: Sample mass, g;

Cpr: Sample protein concentration, mg/mL;

N: Number of cells, count by 10^4.

Note:

If A_T>1.5, it is suggested that the sample should be diluted with distilled water for determination, and the calculation formula should be multiplied by the dilution multiple synchronously.