

## Urea Nitrogen (BUN) Assay Kit

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

**Operation Equipment:** Spectrophotometer

**Catalog Number:** AK0424

**Size:**50T/24S

### Components:

Reagent I: Powder×2. Storage at 4°C . Add 5 mL of distilled water to per bottle before use, fully dissolved. Prepared when the solution will be used.

Reagent II : Solution 15 mL×1. Storage at 4°C .

Reagent III: Solution A, 3 mL×1. Solution B, 12 mL×1. Storage at 4°C . Working solution: Mix Solution A with Solution B (1:4). Prepare when the solution will be used.

Reagent IV: Solution 15 mL×1. Storage at 4°C .

Standard: Powder×1. Storage at 4°C . 10 mg urea. Dissolve with 4.66 mL distilled water to form 1 mg/mL urea standard solution.

### Product Description

Urea (BUN) is the main product of human protein metabolism. Urea constitutes the majority of non-protein nitrogen in blood. Blood urea nitrogen is one of the main indexes of renal function. This kit use indophenol blue colorimetric method to test  $\text{NH}_3$ -N product by urease hydrolysis. The concentration of indophenol is proportional to urea nitrogen concentration.

### Reagents and Equipment Required but Not Provided:

Spectrophotometer, balance, cryogenic centrifuge, 1 mL glass cuvette, mortar/homogenizer, constant temperature water bath pot.

### Procedure:

#### I. Sample preparation:

1. Tissue sample

Suggested 0.1 g tissue with 1 mL distilled water. Fully grind on ice, 12000 g 4°C centrifuge for 15 min.

Take supernatant on ice for test.

## 2. Cells

Collect cells into centrifuge tube, suggested 5 million with 1 mL distilled water. Use ultrasonication to splitting cells (placed on ice, 300W, work time 3s, interval 7s, total 3 min). 12000 g 4°C centrifuge for 15

min. Take supernatant on ice for test.

## 3. Serum (plasma) sample:

Detect sample directly.

## II. Determination procedure:

1. Preheat the spectrophotometer 30 min, adjust the wavelength to 630 nm and set zero with distilled water.

2. Standard solution: dilute urea standard solution (1 mg/mL) with distilled water to 25 µg/mL.

3. Add reagents with the following list:

Reagent Name (µL)	Blank Tube (Ab)	Standard Tube (As)	Test Tube (At)	Control Tube (Ac)
Sample			60	60
Standard Solution		60		
Distilled water	60			120
Reagent I	120	120	120	
Reagent II	220	220	220	220
Mix well, place at 37°C for 10 min.				
Reagent III	80	80	80	80
Reagent IV	60	60	60	60
Mix well, place at room temperature for 30 min.				
Distilled water	460	460	460	460
Mix well, detect absorbance at 630 nm. $\Delta A_s = A_s - A_b$ , $\Delta A_t = A_t - A_c$ .				

### III. Calculation:

1. Calculated by sample weight

$$\text{Urea Nitrogen content } (\mu\text{g/g}) = \Delta A_t \div \Delta A_s \times C_s \times V_e \div W = 25 \times \Delta A_t \div \Delta A_s \div W$$

2. Calculated by protein concentration

$$\text{Urea Nitrogen content } (\mu\text{g/mg}$$

$$\text{prot}) = \Delta A_t \div \Delta A_s \times C_s \times V_e \div (C_{pr} \times V_e) = 25 \times \Delta A_t \div \Delta A_s \div C_{pr}$$

3. Calculated by cell amount

$$\text{Urea Nitrogen content } (\mu\text{g}/10^4 \text{ cell}) = \Delta A_t \div \Delta A_s \times C_s \times V_e \div n = 25 \times \Delta A_t \div \Delta A_s \div n$$

4. Calculated by liquid volume

$$\text{Urea Nitrogen content } (\mu\text{g/mL}) = \Delta A_t \div \Delta A_s \times C_s = 25 \times \Delta A_t \div \Delta A_s$$

Cs: concentration of standard working solution, 25  $\mu\text{g/mL}$ ;

Ve: extraction volume, 1 mL;

W: sample weight, g;

Cpr: sample protein concentration, mg/mL;

n: cell amount.  $10^4$ .

#### Note:

1. Reagent I working solution can be stored at 2-8°C for one week.
2. If measured value of  $\Delta A$  or  $A_t$  exceed 1, it is suggested dilute sample with distilled water for determination.

#### Technical Specifications:

Minimum Detection Limit: 0.000086  $\mu\text{g/mL}$

Linear Range : 0.390625-50  $\mu\text{g/mL}$

#### Recent Product citations:

[1] Xiaoguang Zhu, Jun Shi, Huicong li, et al. PVT1 knockdown alleviates vancomycin-induced acute kidney injury by targeting miR-124 via inactivation of NF- $\kappa$ B signaling. RSC advances. September 2018;(IF3.049)



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AK0301/AK0300 Nitrate Reductase(NR) Activity Assay Kit

AK0436/AK0435 Glutaminase (GLS) Assay Kit