# Urea Nitrogen (BUN) Assay Kit

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

**Operation Equipment:** Spectrophotometer/microplate reader

Catalog Number: NA0504

Size:100T/48S

#### **Components:**

Reagent I: Powder×2. Storage at 4°C and protect from light. Working solution: add 5 mL 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 10 mL×1. Storage at 4°C and protect from light.

Standard: Powder×1. Storage at 4°C, 10 mg urea. Dissolved with 4.66 mL distilled water, to 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 NH<sub>3</sub>-N product by urease hydrolysis. The concentration of indophenol is proportional to urea nitrogen concentration.

#### Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, balance, cryogenic centrifuge, mortar/homogenizer, micro glass cuvette/ 96-well flat-bottom plate, 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, 13000 g 4°C centrifuge for 15 min. Take supernatant 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). 13000 g 4°C centrifuge for 15 min. Take supernatant for test.

## 3. Serum (plasma) sample:

Detect sample directly.

## II. Determination procedure:

- 1. Preheat the spectrophotometer/microplate reader 30 min, adjust the wavelength to 630 nm 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			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°C for 10 min.						
Reagent III	120	120	120	120		
Reagent IV	90	90	90	90		
Mix well, place at room temperature for 30 min.						
Distilled water	90	90	90	90		
Mix well, detect absorbance at 630 nm. ΔAs=As-Ab, ΔAt=At-Ac.						

#### III. Calculation:

1. Calculated by sample weight

Urea Nitrogen content( $\mu g/g$ )=  $\Delta At \div \Delta As \times Cs \times Ve \div W = 25 \times \Delta At \div \Delta As \div W$ 

2. Calculated by protein concentration

Urea Nitrogen content( $\mu$ g/mg prot)= $\Delta$ At÷ $\Delta$ As×Cs×Ve÷(Cpr×Ve)=25× $\Delta$ At÷ $\Delta$ As÷Cpr

3. Calculated by cell amount

Urea Nitrogen content( $\mu g/10^4$  cell)=  $\Delta At \div \Delta As \times Cs \times Ve \div n = 25 \times \Delta At \div \Delta As \div n$ 

4. Calculated by liquid volume

Urea Nitrogen content ( $\mu g/mL$ )= $\Delta At \div \Delta As \times Cs = 25 \times \Delta At \div \Delta As$ 

Cs: concentration of standard working solution, 25 µg/mL;

Ve: extraction volume, 1 mL;

W: sample weight, g;

Cpr: sample protein concentration, mg/mL;

n: cell amount. 10<sup>4</sup>.

#### Note:

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

#### **Technical Specifications:**

Minimum Detection Limit: 0.00009 ug/mL

Linear Range: 0.78125-100 ug/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)

## **Related products:**

NA0865/NA0622 Nitrate Reductase(NR) Activity Assay Kit

NA0754/NA0512 Glutaminase (GLS) Assay Kit