



Blood Urea Nitrogen Microplate Assay Kit User Manual

Catalog # CAK1218

(Version 1.2A)

Detection and Quantification of Blood Urea Nitrogen (BUN) Content
in Serum, Plasma and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION.....	2
II. KIT COMPONENTS.....	3
III. MATERIALS REQUIRED BUT NOT PROVIDED.....	3
IV. SAMPLE PREPARATION.....	4
V. ASSAY PROCEDURE.....	5
VI. CALCULATION.....	6
VII. TYPICAL DATA.....	7
VIII. TECHNICAL SUPPORT.....	7
IX. NOTES.....	7

I. INTRODUCTION

Blood urea nitrogen (BUN) is a blood test performed as a marker of kidney function. It is part of the basic or comprehensive metabolic panel done for health screening. It is also used to monitor the progression of kidney failure. BUN is produced by the breakdown of protein and usually cleared from the blood by the kidneys. A higher than normal value may show impaired kidney function.

Blood Urea Nitrogen Microplate Assay Kit is designed to measure blood urea nitrogen directly in biological samples without any pretreatment. The intensity of the color, measured at 525nm, is directly proportional to the blood urea nitrogen concentration in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	12 ml x 1	4 °C
Dye Reagent	Powder x 1	4 °C
Standard	Powder x 1	4 °C
Technical Manual	1 Manual	

Note:

Dye Reagent: add 6 ml distilled water to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use; then add 0.01 ml into 0.99 ml distilled water. The concentration will be 1 mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 525 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Convection oven

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube for detection.

3. For liquid samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Sample	20 μ l	--	--
Standard	--	20 μ l	--
Distilled water	--	--	20 μ l
Reaction Buffer	120 μ l	120 μ l	120 μ l
Dye Reagent	60 μ l	60 μ l	60 μ l
Mix, put the plate into the convection oven, 90 °C for 20 minutes. When cold, record absorbance measured at 525 nm.			

Note:

- 1) Perform 2-fold serial dilutions of the top standards to make the standard curve.
- 2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.
- 3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the protein concentration of sample

$$\begin{aligned} \text{BUN } (\mu\text{mol/mg}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / \\ &\quad (V_{\text{Sample}} \times C_{\text{Protein}}) \\ &= (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

2. According to the quantity of cells or bacteria

$$\begin{aligned} \text{BUN } (\mu\text{mol}/10^4 \text{ cell}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / \\ &\quad (V_{\text{Sample}} \times N / V_{\text{Assay}}) \\ &= (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / N \end{aligned}$$

3. According to the weight of sample

$$\begin{aligned} \text{BUN } (\mu\text{mol/g}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / (V_{\text{Sample}} \\ &\quad \times W / V_{\text{Assay}}) \\ &= (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / W \end{aligned}$$

4. According to the volume of sample

$$\begin{aligned} \text{BUN } (\mu\text{mol/ml}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / \\ &\quad V_{\text{Sample}} \\ &= (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) \end{aligned}$$

V_{Sample} : the volume of sample, 0.01 ml;

V_{Standard} : the volume of standard, 0.01 ml;

V_{Assay} : the volume of Assay buffer, 1 ml;

C_{Standard} : the standard concentration, 1 mmol/L = 1 $\mu\text{mol/ml}$;

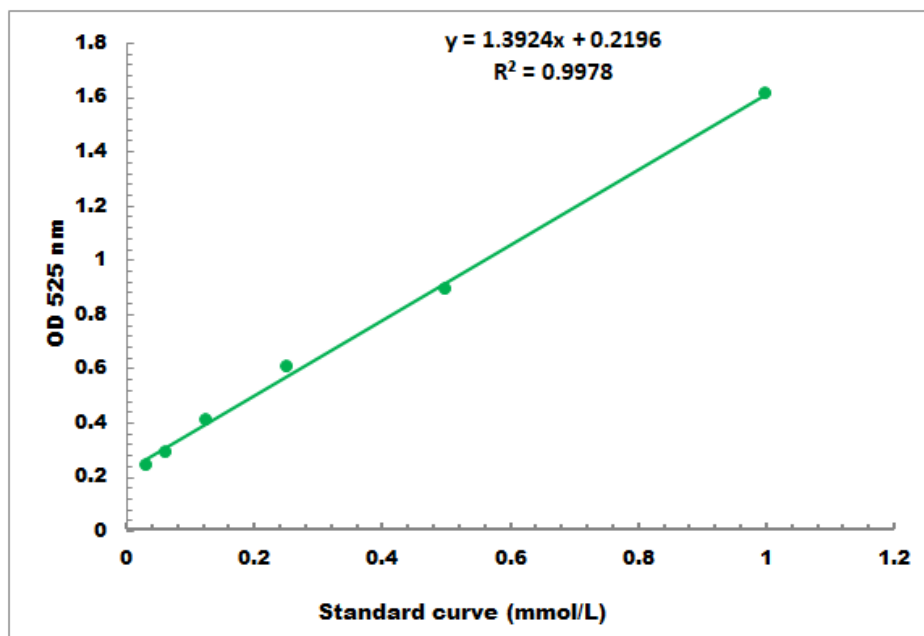
C_{Protein} : the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.05 mmol/L - 1 mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES