Blood Urea Nitrogen (BUN) Assay Kit

(Cat/No.:BC057 Size: 100T/96S)

1. Principle (Diacetyl Oxime Colorimetric Method)

Under heating and strong acid conditions, urea nitrogen reacts with diacetoimide to form a red aziridine, known as the Fearon reaction. The color intensity can be used to determine the urea nitrogen content.

2. Specimens

Plasma anticoagulated with oxalate, heparin, or EDTA. Plasma urea nitrogen remains stable at room temperature for 24 hours and at 4~6°C for at least 7 days. Urine is diluted with normal saline at a ratio of 1:10 to 1:50 and processed as the same as plasma. If the concentration exceeds the linear range, further dilution is required.

3. Composition (The kit is valid for 1 year)

Reagent I: 100mL of 1 g/L oxime solution (1 bottle), stored at 4°C.
Reagent II: 40mL of acid solution per bottle. Dilute with 80mL of distilled water (or mix in a 1:2 ratio) to prepare the acid application solution, and store at 4°C.
Reagent III: 1 vial of 10 mmol/L urea nitrogen standard solution, stored at 4°C.

4. Required Instruments and Reagents

Adjustable 520nm ultraviolet spectrophotometer and cuvette (or microplate reader (520nm) and 96-well plate), vortex mixer, water bath (95~100°C), test tube or centrifuge tube, distilled water, stopwatch, pipettes of various specifications.

5. Procedures

	Blank tube	Standard tube	Measure tube
distilled water (mL)	0.02		
10 mmol/L urea nitrogen standard (mL)		0.02	
Sample to be tested (mL)			0.02
Reagent I (mL)	1	1	1
Reagent II Acid solution (mL)	1	1	1
Mix well, place in boiling water for an accurate 15-minute water bath, remove and cool with tap water, mix again, measure the absorbance value A of each tube at a wavelength of 520nm (or take 200 μL of the reaction solution from each tube and add it to a 96-well plate, and read the value at 520nm with an enzyme detector)			

6. Calculation formula

$$\text{BUN volume (mmol/L)} = \frac{A_{measure} - A_{blank}}{A_{standard} - A_{blank}} \times C_{standard} \times N$$

$C_{standard}$: The standard substance concentration is 10 mmol/L (280.1 mg/L).

N: Dilution factor before sample testing.

7. Notes

1. Acid solution and oxime solution can be mixed in equal amounts, with a dosage of 2 mL, but this mixture can only be stored for approximately 7 days.

2. If precipitation is observed before colorimetry, centrifuge at 3500 rpm for 10 minutes.

3. When the measured OD is excessively high (greater than 0.8), the sample should be appropriately diluted, and the result should be multiplied by the dilution factor.

4. Specimens with severe lipemia should be tested using protein-free filtrate.

5.The reagent shall be stored at 4°C with a shelf life of one year.

6. This method can not only be read using a spectrophotometer, but also involve drawing an appropriate amount of the reaction mixture after completion and adding it to a 96-well plate (note: avoid introducing bubbles). The absorbance value is then measured at 520nm with an enzyme-labeled instrument, and the result is substituted into the formula for calculation.

Appendix: Standard Curve Preparation for Urea Nitrogen (Optional)

1. Preprocessing:

Take 20 mmol/L urea nitrogen standard (not included in this kit) and dilute it with distilled water to the following concentrations: 20 mmol/L, 15 mmol/L, 10 mmol/L, 8 mmol/L, 5 mmol/L, 4 mmol/L, 2 mmol/L, 1 mmol/L, and 0.5 mmol/L for preparing the standard curve.

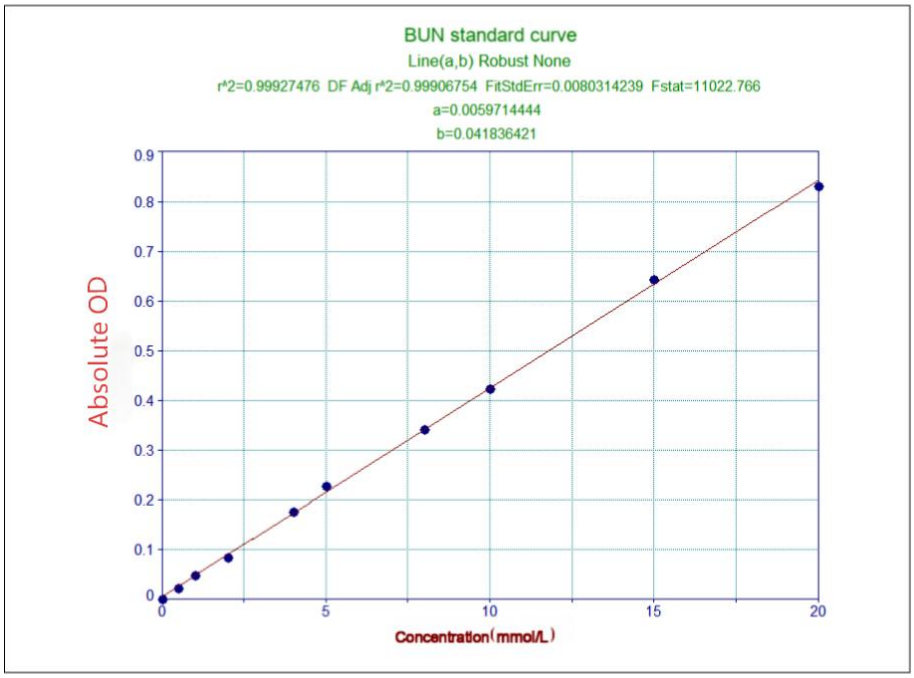
2. Operation Table:

	Blank tube	Standard tube
Distilled water (mL)	0.02	
Urea nitrogen standard solutions at different concentrations (mL)		0.02
Reagent I (mL)	1	1
Reagent II Acid solution (mL)	1	1
Mix well, place in boiling water for an accurate 15-minute water bath, immediately cool with tap water, set at a wavelength of 520nm, with a 1cm optical path, zero the spectrophotometer with distilled water, and measure the absorbance values A of each tube.		

3. Test results:

Standard solution concentration (mmol/L)	OD value	Absolute OD value
0	0.012	0
0.5	0.034	0.022
1	0.060	0.048
2	0.096	0.084
4	0.188	0.176
5	0.240	0.228
8	0.355	0.343
10	0.436	0.424
15	0.656	0.644
20	0.843	0.831

4. The drawing is as follows:



Note: The above standard curve can be omitted by users. Simply follow the previous procedure table for measurement and calculate according to the formula, with no impact on the results.