ELK Biotechnology

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Blood Urea Nitrogen Assay Kit

CAT/NO.: BC027 Urease Methods

I. Significance of Measurement

Urea as the final product of the protein metabolism process, is the main component of the non-protein nitrogen. Blood urea nitrogen (BUN) which is mainly produced from liver is normally excreted by urine. Patients suffering renal failure, nephritis, and urinary tract obstruction may have higher BUN levels than normal.

II. Principle of Measurement

In presence of urease, urea can be hydrolyzed to ammonium anion and carbon dioxide. Ammonium in alkaline solution can generate blue product with chromogenic agent. The optical density (OD) at 640 nm is directly related to the urea concentration and thus can be calculated.

III. Sample Requirement

Plasma with anticoagulant like oxalate, heparin or EDTA. Blood urea nitrogen (BUN) is stable at room temperature for 24 hours and at 4-6°C for at least 7 days. Urine sample can be diluted to 10-50 times of its initial volume before the measurement and further dilution can be made were the results exceed the range allowed for the measurement.

IV. Reagent Compositions and Preparation

Reagent I: Enzyme stock solution: 1 Bottle×0.1ml. Diluent: 1 Bottle×30ml. Preserved at 4°C.
Preparation of Reagent I Solution: Dilute the stock solution with diluent with the ratio of 3:1000.
Reagent II: Chromogenic Agent: 1 Bottle×100ml. Preserved at 4°C in darkness.
Reagent III: Sodium Hypochlorite Solution. 1 Bottle×100ml. Preserved at 4°C.
Reagent IV: BUN Standard. 3 Bottles of Urea Powder. 6.006 mg each.

Preparation of 100mM BUN Stock Solution: Dissolve each bottle of powder with double distilled water (DDW) to 1 ml. Solution should be preserved at 4°C.

Preparation of 10mM BUN Solution (Reagent IV Solution): Dilute the stock solution with

DDW at the desired rate.

V. Procedures

Compositions (ml)	Blank	Standard	Sample
DDW	0.02		
Reagent IV Solution		0.02	
Sample			0.02
Reagent I Solution	0.25	0.25	0.25
Mix thoroughly and warm the mixture in a water bath at 37°C for exactly 10 min.			
Reagent II	1	1	1
Reagent III	1	1	1

Mix thoroughly and warm the mixture at 37° C in a water bath for 10 min. Zero the 1 cm path length cuvettes at 640 nm with DDW and record the OD value of each tube.

VI. Calculation Formula and Example

1. Formula

$$\underset{mM}{BUN\ Conc.} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \underset{10mM}{C_{Standard}} \times \underset{Pretreatment}{CoD}$$

CoD represents the coefficient of dilution in the pretreatment process.

2. Example

20 μl human serum was measured with OD values equal to 0.018, 0.250 and 0.158 respectively.

$$\underset{mM}{BUN\ Conc.} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \underset{10mM}{C_{Standard}} \times \underset{Pretreatment}{CoD} = \frac{0.158 - 0.018}{0.250 - 0.018} \times 10 \times 1$$
$$= 6.034mM$$

3. Reference value

BUN concentration in human serum is about 2.9-8.2 mM.

VII. Notes

1. Dilution can be made were the results too high.
2. Disposable tubes are recommended to avoid contamination.

3. Reagent I solution should be prepared prior to the measurement as its unstability at RT.
4. Enzyme stock solution is Viscous and thus the addition should be low to avoid clinging to the tips.
5. The solution should be warmed for 10 min after the addition of reagent I solution. Were the number of samples too high, measurement should be done in batches with no more than 15 samples per batch.
6. The color generated is stable within 4 hours.
7. The quality control can be done with known serum samples which are available by the institute.