UREA (BUN) - BERTHELOT

METHOD – UREASE - BERTHELOT
PRODUCT CODE – DU01

ANAMOL
THE ORIGINAL MAKERS

INSTRUCTIONS FOR USE

INTENDED USE: Test for estimation of Urea in serum / plasma using Urease / Chromogen method.

SUMMARY AND PRINCIPLE

Increased Urea levels are associated with renal diseases, as well as dehydration, gastrointestinal haemorrhage and diabetic coma. Decreased values are observed in some cases of severe liver disease. Urea is a reagent set for quantitative determination of Urea in human serum / plasma based on enzymatic method, using Urease. Urea is a two-reagent system, using two step procedure.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + \text{Hypochlorite} + \text{Phenolic Chromogen} \longrightarrow \text{Green Colour}$$

KIT COMPONENTS

Reagent 1: Enzyme powder
Reagent 2: Diluent Reagent
Reagent 3: Chromogen Solution
Reagent 4: Urea Standard (50 mg/dL)

REAGENT PREPARATION, STORAGE & STABILITY

Reconstitute reagents as per instructions on individual bottle label to prepare working reagent. Mix by gentle swirling or inversion. Do not shake vigorously. The reconstituted enzyme solution is stable for 1 month when stored at 2-8 ^{0}C. The chromogen is ready to use and is stable till expiry when stored at 2-8 ^{0}C but once opened the chromogen is stable for six months. The reagent kit should be stored at 2- 8 ^{0}C and is stable till the expiry date indicated on the label.

PRECAUTIONS & HANDELING

The reagents/samples should be handled by qualified personnel only. Discard reagent/sample as per good laboratory practices and local regulatory requirements. Read the instructions given on the labels and instructions for use carefully before using the kit. The kit is intended for in-vitro diagnostic use only. Don't freeze the reagent. Do not shake the reagent vigorously. Discard the reagent if the absorbance of the reagent exceeds 0.200 O.D. against D/W at 578 nm. Contamination of the reagent should be avoided.

TEST PARAMETERS

Name	Urea
Reaction Type	End Point
Wavelength Primary	578 nm
Flow Cell Temp.	37 °C
Blank setting	Reagent
Blank Abs Limit	<0.200
Linearity	350 mg/dL

Enzyme Reagent Volume	1000 µl
Sample Volume	10 µl
1st Incubation Time	5 mins
Chromogen Reagent Volume	1000 µl
2nd Incubation Time	5 mins
Incubation Temp.	37 °C
Standard Conc.	50 mg/dL

MATERIALS REQUIRED BUT NOT PROVIDED

Test tubes, Micropipette with tips, Analyzer, Controls, Incubation chamber.

SPECIMEN COLLECTION & PRESERVATION

Blood should be collected in a clean dry container (free of NH_4+). Ammonium salt of anticoagulants and sodium fluoride should not be used as anticoagulant. Heparinized or EDTA plasma can be used. Urea in the specimen is stable for a week when stored at 2-8 ^{0}C and for 6 months when stored at -10 ^{0}C.

COMPONENTS OF REAGENT

Component	Concentration
Phosphate Buffer, pH 7.0	35 mmol/l
Urease	7.5 KU/L
Phenolic Chromogen	2 mmol/l
Hypochlorite	4 mmol/l
Stabilizers and inactive ingredients.	

ASSAY PROCEDURE

	Blank	Standard	Test
Enzyme Reagent	500 µl	500 µl	500 µl
Standard		5 µl	
Sample			5 µl
1st Incubation: Mix the reagent and sample/standard in the above-mentioned ratio and incubate for 5 mins at 37 ^{0}C			
Chromogen	500 µl	500 µl	500 µl
2nd Incubation: Add chromogen in above mentioned volume and incubate the assay mixture for 5 mins at 37 ^{0}C			
Aspirate reaction mixture into flow cell and measure the absorbance.			
The final colour is stable for 2 hours if not exposed to light.			

CALCULATION

$$\text{Urea (mg/dL)} = \frac{\text{Abs. of sample x 50}}{\text{Abs. of standard}}$$

$$\text{Urea Nitrogen (mg/dL)} = \frac{\text{Urea (mg/dL)}}{2.14}$$

Measuring Range: The assay is linear between 2.5 - 350 mg/dL. If the Urea value exceeds linearity limit (above 350 mg/dL), dilute the specimen suitably with normal saline and repeat the assay. In that case, assay value should be multiplied with the dilution factor to obtain correct Urea value of the specimen.

Interference: There is no significant interference in samples containing Bilirubin upto 20 mg/dL and Haemoglobin upto 500 mg/dL.

Precision: Precision studies has been carried out using quality control sera as shown below:

(n=10)	Within Run			Between Run		
Specimen Material	Mean (mg/dL)	SD (mg/dL)	CV %	Mean (mg/dL)	SD (mg/dL)	CV %
Low Value Serum	33.9	0.71	2.1	29.87	0.64	2.2
High Value Serum	97.13	1.19	1.2	106	1.29	1.2

Note: We recommend all the laboratories to establish its own accuracy and precision data.

QUALITY CONTROL

Inclusion of a normal value and abnormal value chemistry control serum in each test run ensures optimum quality control. Consistent use of same type and methodology of control serum provides between run precision and accuracy data for Urea. We recommend to produce such data on daily basis for greater accuracy in assay system which include reagents, instrument, apparatus and operator.

PRECAUTIONS

1. Discard the working reagent if its absorbance exceeds 0.200 at 578 nm against distilled water.
2. Fluoride as an anticoagulant cannot be used as it inhibits Urease activity.
3. Anticoagulant having ammonium ions should not be used because of extreme sensitivity of the colour reaction to ammonia.
4. If the Urea value exceeds 350 mg/dL, dilute the specimen and carry out the assay. In such case multiply the result obtained with the dilution factor to obtain correct Urea value.
5. Detergent containing ammonium ions and strong oxidising disinfectant (sodium hypochlorite) should not be used for washing glassware.

BIBLIOGRAPHY

1. Webster D., Clin. Chem.23, 663 (1977).
2. Dumas B.T.; et al, Clin Chem. Acta, 31,87 (1971).

Symbol	Explanation	Symbol	Explanation
	Manufactured By		In Vitro Diagnostic Use
	Lot Number		Read Instructions Before Use
	Catalogue Number		Storage Temperature
	Manufacturing Date		Number of Tests / Volume
	Expiry Date		Do Not Reuse
	Protect from Sunlight		Keep Dry

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ISO 9001: 2015
ISO 13485 : 2016
GMP
CE