Pariksha Biotech
A game changer in IVD

Urea Estimation Kit

High-Q Urea

Modified Berthelot Method

HIGH®
Command on Quality

Intended Use:

Kit for the quantitative determination of urea in human serum, plasma and urine.

Summary & Clinical Significance:

The determination of urea is the most widely used test for the evaluation of kidney function. The test is frequently used in conjunction with the determination of creatinine for the differential diagnosis of prerenal hyperuremia (cardiac decompensation, water depletion increased protein catabolism), renal hyperuremia (glomerulonephritis, chronic nephritis. polycystic kidney, nephrosclerosis, tubular necrosis) and postrenal hyperuremia (obstructions of the urinary tract). Urea is the final degradation product of protein and amino acid metabolism. In protein catabolism the proteins are broken down to amino acids and deaminated. The ammonia formed in this process is metabolized to urea in the liver. This is the most important catabolic pathway for eliminating excess nitrogen in the human body. In 1914 Marshall introduced an assay based on the enzyme urease for determining urea in blood. The ammonia released from urea by urease was measured titrimetrically. Numerous other techniques have since been employed to measure the ammonia produced. These include Berthelot's indophenol assay and the reaction of ammonia with Nessler's reagent.

Principle:

Urease catalyses the conversion of Urea to Ammonia and Carbondioxide. The ammonia released reacts with a mixture of Salicylate, Hypochlorite and Nitroprusside to yield a blue-green colored compound (Indophenol).

The intensity of color produced is proportional to the concentration of urea in the sample and is measured photometrically at 578 nm.

$$Urea + H2O \xrightarrow{Urease} 2NH3 + Co2$$

$$Nh3 + Salicylate + Hypochlorite \xrightarrow{Nitroprusside} 2 - 2 - Dicarboxy\ Indophenol.$$

Normal Ranges:

Serum / Plasma Urea	:	10-50 mg/dl
Urine Urea	:	25-43 gm/24 hrs
Serum / plasma Urea Nitrogen	:	5-23 mg/dl

It is recommended that the laboratories should establish their own normal range.

Working Reagent Composition:

Urease = 40,000 U/l
Sodium Hypo chlorite =30 mMol/l
NaOH = 380 mMol/l
Sodium Salicylate = 50 m mol/l
Sodium Nitroprusside = 28 m mol/l
Activators and Stabilizers

Storage and Stability:

All the reagents must be stored at 2-80C and are stable till the expiry date mentioned on the labels.

Notes:

1. Urease Reagent is pale yellow in color due to which the Blank absorbance may read upto 0.200 at 578 m against distilled water. The absorbance of Standard and Test read against Reagent Blank at 578 nm nullifies the absorbance of Urease Reagent.
2. Contamination of reagents and standard after opening must be avoided. After use, all the reagents must be immediately stored back at 2-8^{0}C. Working Reagent is stable for 12 months at 2-8°C.
3. No interference was observed by ascorbic acid up to 32 mg/dl, bilirubin up to 43 mg/dl, hemoglobin up to 500 mg/dl and lipemia up to 2200 mg/dl triglycerides.
4. If the urea value exceeds 350 mg/dl, dilute sample with normal saline. In such a case, the result obtained must be multiplied with the appropriate dilution factor.
5. Working reagent preparation is a very important step and the instructions need to be followed Strictly.

Specimen:

Serum / Heparinised or EDTA Plasma (do not use ammonium salts and sodium fluoride as anticoagulants) Urine (dilute 1:100 with distilled water).

Working Reagent Preparation : (Very important instructions to be followed strictly)

1. Transfer the entire Enzyme Concentrate into Urease Reagent with the fresh new microtip.
2. After transferring the Enzyme Concentrate in to the Urease Reagent rinse the Enzyme Concentrate Vial thoroughly at least three times with Urease Reagent and take the left over enzyme completely to ensure proper reconstitution as the enzyme is provided in the highly concentrated form.
3. The reconstituted reagent is stable for 12 months when proper storage conditions are strictly maintained.
4. Avoid keeping the reconstituted reagent at room temperature for a long time.
5. It is advised to keep the reagent back at 2-8°C once the assay is over.
6. Slight haziness / turbidity may appear in the Enzyme concentrate and is only due to high concentration of enzyme. Slight haziness / turbidity of enzyme concentrate disappears once it is added to Urease Reagent and does not affect test performance and results.

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System Parameters:

Reaction type	:	End Point
Flow cell Temp	:	37^{0}C
Units	:	mg/dl
Reagent Volume	:	2000 µl (1000 µl R1,1000 µl R2)
Sample Volume	:	10 µl
Wavelength	:	578 nm (570-620 nm)
Blank	:	Reagent
Standard Conc	:	50
Linearity	:	350
High Normal	:	50
Low Normal	:	10

Procedure::

Reagent	B	S	T
Working Reagent	1.0 ml	1.0 ml	1.0 ml
Urea Standard (Conc. 50 mg/dl)	----	10 µl	----
Specimen	----	----	10 µl
Mix and incubate for 5 minutes at 37°C (5 min. at R.T.)			
Alkaline Reagent	1.0 ml	1.0 ml	1.0 ml
Mix and incubate for 5 minutes at 37°C (10 min. at R.T.)			

Mix and read absorbance of Standard (S) and Test (T) against Blank (B) at 578 nm (570-620 nm).
The final color is stable for 10 hours at RT.

Calculations:

(a) Serum / Plasma Urea in mg/dl = $\frac{\text{Abs. of T}}{\text{Abs. of S}}$ x 50

(b) Blood Urea Nitrogen (BUN) in mg/dl = a x 0.467

© Urine Urea in gm / 24 hours = a x 24hrs urine volume in litres
Urine UREA/BUN in gm/24hours = Conc. of UREA in gm/L x 24 hours Urine Collected in Liters.

Estimantion of UREA /BUN in Urine (gm/24 hours)

Procedure:

Measure and record 24 hrs urine volume collected in liters.
Determine the UREA/ BUN Conc. in mg/dl using High-Q Urea(Berthelot) kit.
Convert the UREA/BUN Conc. into mg/L by multiplying with factor “10”.
Convert the UREA/BUN Conc. from mg/L to gm/L by dividing with “1000”.
Multiply the UREA/BUN conc. which is in gm/L with 24 hrs urine collected in liters to get the UREA/BUN Conc. in gm/24hrs.

eIFU Indicator

Pariksha's world inside
SCAN TO EXPLORE MORE

Manufactured in India by :
Pariksha Biotech Pvt Ltd,
Plot no.1/B-14, SVICE,
Balanagar,
Hyderabad-500037
Telangana State

Impreciosion:

Serum
Reproducibility was determined using human samples and controls between day (n = 20). The following results were obtained:

	Within run		
Sample	Mean mg/dl	SD mg/dl	CV %
Sample 1	39.61	0.88	2.21
Sample 2	90.95	5.65	6.21
Sample 3	139.78	1.95	1.40

Reproducibility was determined using human samples and controls between day (n = 18). The following results were obtained:

	Between day		
Sample	Mean mg/dl	SD mg/dl	CV %
Sample 1	39.85	1.52	3.81
Sample 2	89.48	3.47	3.87
Sample 3	140.20	5.27	3.76

References:

- Chaney, A.L. and Marbach, E.P. (1962) Clin. Chem. 8, 130
- Tietz NW. Fundametals of Clinical Chemistry Philadelphia, Pa: WB Saunders Co 1976:991 .

Ordering information:

Cat No:	Pack Size	Presentation
P-URE (B)-50	2 x 50 ml (50 Det)	Two Liquid Reagents and Enzyme Concentrate
P-URE (B)-100	2 x 100 ml (100 Det)	
P-URE (B)-200	4 x 100 ml (200 Det)	

Product Features

- **Liquid Stable, Ready to use Two Reagents with Enzyme Concentrate**
- **Working reagent stability of 12 months at 2-8°C.**
- **10 Minutes End Point Assay (5 Min + 5 Min)**
- **Lipid Clearing Factor**
- **Linearity 350 mg/dl**
- **Measuring Wavelength 578 nm (570-620)**
- **Aqueous Urea Standrad provided (Standrad Conc: 50 mg/dl)**
- **BUN values can be estimated**
- **Serum/ Heparinized or EDTA Plasma/ Diluted Urine as specimens**
- **Available as multipurpose reagents.**

Symbols used with IVD devices

	Date of manufacture		Manufactured by
IVD	In vitro diagnostic device		Keep away from sunlight
	Do not freeze		This way up
	Use by (yyyy-mm-dd or mm/yyyy)	R	Reagent
CAL	Calibrator Material	LOT	Batch code
2°C 8°C	Temperature limitation (store at)	CONTROL	Control
	Consult instructions for use		Keep dry Keep away from rain
REF	Catalog Number		

+91 7075706709 info@parikshabio.com www.parikshabio.com

AN ISO 13485 Certified Company

Rev # 2