UREA UV TEST KIT

INTENDED USE : This Reagent kit is intended for ***"In Vitro"*** quantitative determination of Urea concentration in serum & Urine. Enzymatic (UV) Method.

CLINICAL SIGNIFICANCE : The detoxification of NH4+ formed in the catabolism of amino acids takes place in the Urea cycle. Enzymes catalyzing these reactions are synthesized in the liver. The end product is Carbamide (Urea) which is a nontoxic, nonpolar, small molecule. It is eliminated by the kidney. Increased levels are associated with renal diseases, as well as dehydration, circulatory collapse gose, gastrointestinal hemorrhage and diabetic coma. Decreased values are observed in some cases of severe liver disease.

PRINCIPLE : Ammonia and Carbon dioxide (CO_2) are produced when urea is hydrolyzed in presence of Urease. The Ammonia produced in the reaction combines with 2 - Oxoglutarate and NADH in the presence of Glutamate dehydrogenase (GLDH) to yield glutamate and NAD^+. The NADH/NAD^+ reaction produces a unique change in absorbance at 340 nm, which correlates with the concentration of urea nitrogen in the sample.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + Co_2$$

$$2NH_4^+ + 2\text{ - Oxoglutarate} + 2NADH \xrightarrow{\text{GLDH}} 2\text{L - Glutamate} + 2NAD^+ + 2H_2O$$

REAGENT COMPOSITION :
Reagent 1 : Enzyme Reagent
Reagent 2 : Substrate Reagent
Urea Standard : 40 mg/dl

MATERIALS REQUIRED BUT NOT PROVIDED :
- Clean & Dry Glassware.
- Micropipettes & Tips.
- Colorimeter or Bio-Chemistry Analyzer.

SAMPLES : Serum free of hemolysis. Urine diluted in ratio of 1:100 with distilled water. Do not use Anticoagulants containing fluoride or ammonium ions.

STABILITY OF REAGENT : When Stored tightly closed at 2 to 8°C temperature protected from light and contaminations prevanted during their use; reagents is stable up to the expiry date stated on the lable.

Avoid Direct Exposure to Light.

WORKING REAGENT : Mix 4 part of Buffer Reagent with 1 part of Enzyme Reagent. The working reagent is stable for 30 days at 2 - 8°C.

ASSAY PROCEDURE :

	Standard	Sample
Reagent	1000 µl	1000 µl
Standard	10 µl	-
Sample	-	10 µl

Mix well and after 30 secs. incubation read initial absornance A1. Exactly after 60 Secs. interval read absorbance A2. Determine the Δ Absorbance per Minuts Δ Abs. = A2 - A1.

GENERAL SYSTEM PARAMETERS :

Reaction Type	Fixed Time (Decreasing)
Wavelength	340 nm
Light Path	1cm
Reaction Temperature	37°C
Blank / Zero Setting	Distilled Water
Reagent Volume	1000 µl
Sample Volume	10 µl
Delay / Lag Time	30 Seconds
Read Time	60 Seconds
Read Interval	60 Seconds
Standard Concentration	40 mg/dl
Low Normal	15 mg/dl
High Normal	45 mg/dl
Linearity	300 mg/dl

CALCULATION :

$$\text{Urea Conc. (mg/dl)} = \frac{\Delta \text{ Abs. of Sample}}{\Delta \text{ Abs. of Standard}} \text{ X Conc. of standard}$$

LINEARITY : Reagent is Linear up to 300 mg/dl.
Dilute the sample appropriately and re-assay if Urea concentration exceeds 300 mg/dl.
Multiply result with dilution factor.

REFERENCE NORMAL VALUE :
Serum, plasma : 13 - 43 mg/dl
Urine : 26 - 43 g/24 Hrs.

QUALITY CONTROL : For accuracy it is necessary to run known controls with every assay.

SENSITIVITY / LIMIT DETECTION : The Lower Limit of detection is 2 mg/dl.

LIMITATION & PRECAUTIONS :
- Storage conditions as mentioned on the kit to be adhered.
- Do not freeze or expose the reagents to higher temperature as it may affect the performance of the kit.
- Before the assay bring all the reagents to room temperature.
- Avoid contamination of the reagent during assay process.
- Do not use the reagent if it is hazy or cloudy.

BIBLIOGRAPHY :
Teitz. N. W.; Fundamentals of Clinical Chemistry, Philadelphia, W. B. Saunders & Co., Philadelphia, PA, P991 (1976)., Talke H, Schubert GE, Klin Wchers., (1965), 43, 174.

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