ANA BIO ISP UREA

(GLDH method)

For Miura Instruments

Intended Use
Urea is a reagent kit used for the quantitative determination of Urea / **B**lood **U**rea **N**itrogen (BUN), based on enzymatic method using Urease and Glutamate dehydrogenase (GLDH) enzymes.

Principle
Urea is hydrolyzed to ammonia and carbon dioxide by urease. This Ammonia reacts with α-ketoglutarate to form glutamate in presence of glutamate dehydrogenase. NADH is oxidized to NAD^+ in this reaction. The decrease in absorbance due to NADH consumption is proportional to the Urea concentration in the specimen.

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

$$NH_3 + \alpha\text{-ketoglutarate} + NADH \xrightarrow{\text{GLDH}} \text{Glutamate} + NAD^+$$

Components & Concentration of Reagents

Reagent	Component	Concentration
Reagent 1	Goods buffer, pH 7.5	320 mmol/L
	Urease	≥ 7000 U/L
	GLDH	≥ 700 U/L
	Stabilizers, excipients & surface active agents	
Reagent 2	Goods Buffer, pH 9.5	15 mmol/L
	NADH	≥ 0.25 mmol/L
	α –KG	≥ 10 mmol/L
	Stabilizers, excipients & surface active agents	

Reagent storage and stability
The kit should be stored at 2° - 8°C and is stable till the expiry date indicated on the label. **DO NOT FREEZE THE REAGENT.** Contamination of the reagent should be strictly avoided.

Reagent Preparation
Liquid reagents ready for use. After opening the reagents of R1 and R2 are stable for 30 days if closed, stored at 2° - 8°C, and protect from direct light and contamination. Do not mix different batches.

Specimen collection and preservation
Blood should be collected in a clean dry container. Although serum is preferred, plasma with heparin or EDTA can also be used. Anticoagulants such as ammonium heparin and fluoride should not be used. Blood Urea Nitrogen (BUN) concentration in serum / plasma is stable for 6 days at 2° - 8°C and for a month when stored at -20°C. The samples should be brought to room temperature prior to use.

- HEPARIN 200 IU/ml of blood
- SODIUM FLUORIDE 10 mg/ml of blood

Serum/plasma should be separated as quickly as possible from cells. Urea is stable for 4 days at 2° - 8°C and several months when stored at -20°C.

Automation
This kit, though developed and manufactured to be used as manual assay and with I.S.E. Miura Analyzer, can be used also with other analyzers able to meet the specifications indicated in section "Reaction conditions – Test procedure" Application sheets are available for automatic instruments.
All applications not explicitly approved by KDPL. Cannot be guaranteed in terms of performance, and must there be established by the operator.

Calibration
For Calibration use the "Multicalibrator"

Calibration Stability
For the instrumentation series Miura, the calibration is recommended to be done every 10 days.

Materials required but not supplied in the kit
Calibrators and controls

Assay guidelines for Analyzer I.S.E. Miura

Analyte Name	Urea
Method Code	Urea
Type	Fixed Time
Unit	mg/dl
Filter F1	340 nm
Blank in	Not Used

Step	Reaction Volume	U.M.
Volume reagent R1	160	µl
Volume reagent R2	40	µl
Sample Volume	2	µl
Final Incubation	36	Sec.
Kinetic reading time	96	Sec.

Normal range
Guidance value (Urea) : 10 - 45 mg/dl
Urea nitrogen : 5 - 21 mg/dl
Note: Expected range varies from population to population and each laboratory should establish its own normal range.

Limitation
The reagent is linear up to 535 mg/dl Urea and 250 mg/dl of BUN. For higher value, dilute sample with normal saline and perform the assay. Multiply the final result by dilution factor to get the real value.

Quality Control
To ensure adequate quality control, it is recommended that each batch should include a normal and an abnormal commercial reference control serum. It should be realized that the use of quality control material checks both instrument and reagent functions together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glassware, Wavelength setting, Expiration date of reagents and accuracy of prob aspiration.

Accuracy-Recovery
Urea added to a serum matrix containing known amounts of Urea gave an average recovery of 99%.

Interference
There is no significant interference in samples containing upto 60 mg/dl of bilirubin, 700 mg/dl of haemoglobin.

Precision of the Method

Within-run					
Range	U.M	Mean	S.D.	C.V. (%)	No. run
Low	mg/dl	34.5	1.2	3.5	20
High	mg/dl	102.3	3.1	3.0	20
Between run					
Range	U.M	Mean	S.D.	C.V. (%)	No. run
Low	mg/dl	33.9	1.2	3.7	60
High	mg/dl	102.9	3.2	3.1	60

Sensitivity
At 340 nm the sensitivity in terms of detection limit is 4 mg/dl of urea concentration.

References

1. Talke H., Schubert, G.E., Klin. Wochenschr, 43, 174, (1965).
2. Gutman, I., Bergemeyer, H.U., in "Methods of Enzymatic Analysis", H.U. Bergemeyer Ed., Academic Press (1974), p. 1791.
3. Tiffany, T.O., et al, Clin. Chem., 18, 829, (1972).
4. Tietz NW, ed. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, Pa : W.B. Saunders Company, 1995 : 622 – 626.

Symbols

IVD	In Vitro Diagnostics		Caution
LOT	Batch No.		Product Expiry Date
CONT	Content		Manufactured By
	Read Instructions		Date of Manufacture
	Storage Temperature		Keep Dry
REF	Catalogue No.		Fragile
			Keep away from sun light

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