Urease-GLDH Method

In-Vitro Diagnostic reagent for the quantitative determination of Urea present in human serum, plasma or urine samples on photometric system.

ORDER INFORMATION

Cat no.	Kit Information
LG110-100	Reagent R1 - 2 x 40mL Reagent R2 - 2 x 10mL Standard – 1 x 2mL
LG110-1000	Reagent R1 - 2 x 400mL Reagent R2 - 2 x 100mL Standard – 1 x 4mL

SUMMARY

Urea is waste product formed in the liver and filtered out by the kidneys. The increased concentrations of Urea are found in kidney problems, urinary tract obstructions, and congestive heart failures. Its decreased concentrations are observed during hepatic failures and also in pregnant women. Parallel determination of urea and creatinine is performed to differentiate between pre-renal and post-renal azotemia.

PRINCIPLE

Urease enzyme hydrolyses the urea into ammonia and carbon dioxide, this ammonia then further reacts with α-keto glutaric acid. This reaction is catalyzed by Glutamate dehydrogenase (GLDH) NADH and a coloured complex is formed that can be measured by spectrophotometry.

$$Urea + 2H_2O \xrightarrow{Urease} AMMONIA + CO2$$

$$Ammonia + \alpha\text{-ketoglutaric acid} + NADH \xrightarrow{GLDH} L\text{-Glutamate} + NAD^+ + H_2O$$

STORAGE INSTRUCTIONS AND REAGENT STABILITY

Reagent and standard are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C, protected from light and contamination is avoided. Do not freeze the reagents!

REAGENT COMPOSITION

Reagent : Tris 12.20 g/L, α-keto glutaric acid 3.12 g/L, Urease > 10 KU/L, GLDH (Glutamate dehydrogenase) > 1KU/L, Succinic acid 12 g/L, Albumin 1 g/L, NADH 1.10 g/L, Potassium carbonate 2.0 g/L
Standard: Urea - 40 mg/dL

WASTE MANAGEMENT

Please refer to local regulatory requirements.

REAGENT PREPARATION

Mix, 4 parts of reagent 1 with 1 part of reagent 2 = Working reagent.
Working Reagent Stability: 4 weeks at 2°- 8°C.
Protect working reagent from light.

MATERIALS REQUIRED BUT NOT PROVIDED

NaCl solution 9 g/L. General laboratory equipments.

SPECIMEN

- Serum, heparin (not ammonium heparin) or urine.

Stability in serum or plasma: 7 days at 2° - 8° C
3 month at -20°C
Stability in urine: 7 days at 2° - 8°C
1 month at -20°C

For 24-hours urine storage, it should be collected in a thoroughly cleaned container which should be refrigerated during collection, measure diuresis, and take as aliquot and perform a 1:100 dilution with distilled water and calculate the amount of urea eliminated during 24 hours and multiply the results by 100.
Discard contaminated specimens

ASSAY PROCEDURE

Wavelength : 340 nm
Optical path : 10 mm
Temperature : 37 °C

	Blank	Sample or Standard
Working Reagent	1000 µL	1000 µL
Sample or Standard	–	20 µL

Mix, incubate for approx. 30 sec. at 37°C, then read the absorbance (A1). After exactly further 60 sec. read absorbance (A2).
ΔA= (A1-A2) sample/standard

CALCULATION

$$\text{Urea [mg/dL]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Standard}} \text{ X (std conc.)mg/dL}$$

QUALITY CONTROLS

For internal quality control any normal and abnormal controls should be assayed with each batch of samples.
Each laboratory should establish corrective action in case of deviations in control recovery.

WARNINGS AND PRECAUTIONS

- In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- Always use safety pipettes to pull the reagents into a pipette.
- Reagents may contain some non-reactive and preservative components. It is suggested to handle carefully, avoid direct contact with skin and do not swallow.
- The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow. Avoid contact with skin and mucous membranes.
- For professional use only!

PERFORMANCE CHARACTERISTICS

MEASURING RANGE

The test has been developed to determine urea within a measuring range from 5 - 500 mg/dL. When values exceed this range samples should be diluted 1 + 4 with NaCl solution (9 g/L) and the result multiplied by 5.

LINEARITY/LIMIT OF MAXIMUM DETECTION

The maximum limit of detection is 500 mg/dL.

SENSITIVITY/LIMIT OF DETECTION

The lower limit of detection is 5 mg/dL

SPECIFICITY/INTERFERENCES

No interference was observed by, Ascorbic Acid upto 30mg/dL, Bilirubin up to 40 mg/dL, and triglycerides up to 2000 mg/dL.

PRECISION

Intra assay n=20	Mean (mg/dL)	SD (mg/dL)	CV (%)
Sample 1	25.51	0.52	2.03
Sample 2	42.03	0.50	1.20
Sample 3	155.96	1.22	0.78

Inter assay n=20	Mean (mg/dL)	SD (mg/dL)	CV (%)
Sample 1	24.06	0.53	2.19
Sample 2	45.41	0.59	1.31
Sample 3	146.04	0.74	0.51

METHOD COMPARISON

A comparison of Precision Biomed Urea (y) with a Immercially available test (x) using 15 samples gave following results:
y = 1.022x - 0.537; r2 = 0.969

REFERENCE RANGE

In Serum/Plasma		
Adults	**mg/dL**	**mmol/L**
Global	17 - 43	2.8 - 7.2
Men < 50 Years	19 - 44	3.2 - 7.3
Men > 50 Years	18 - 55	3.0 - 9.2
Women < 50 Years	15 - 40	2.6 - 6.7
Women > 50 Years	21 - 43	3.5 - 7.2
Children		
1 - 3 Years	11 - 36	1.8 - 6.0
4 - 13 Years	15 - 36	2.5 - 6.0
14 - 19 Years	18 - 45	2.9 - 7.5
In Urine	26-43 g/24h	0.43-0.72 mol/24h

Note: It is recommended that each laboratory should establish its own reference range based on the patient population.

ASSAY PARAMETERS

Mode	Kinetic fixed time
Reaction slope	Decreasing
Wavelength	340nm
Path length	10mm
Temperature	37°C
Standard conc.	50 mg/dL
Working reagent	4 part Reagent 1 1 Part Reagent 2
Working reagent	1000µL
Sample volume	20 µL
Delay	30 sec.
Rate	60 sec.
Normal range	17 – 43mg/dL
Linearity	500 mg/dL
Sensitivity	5 mg/dL

LITERATURE

- Fawcett, J.K. and J.E. Scott (1960). J. Clin Path. 13:156
- Praful B. Godkar, Text Book of Medical Laboratory Technology, Bhalani Publishing House: pp. 221, 1994.
- Thomas L. Clinical Laboratory Diagnostic. 1ed. Frankfurt: THbooks verlagsgesellschaft; 1998.p.374-7.
- Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p.1838.

INDEX OF SYMBOLS

ISO 13485	International Organization or Standardization		Keep out of Sunlight
	Manufacturer	IVD	For invitro diagnostic use only
	Expiry date		Read product insert before use.
LOT	Lot (batch) number		Do not use if package is damaged
2°C 8°C	Store between 2-8°c		Keep Away From Moisture

ART/IFU/PRC-110-01

Manufactured by:
LABGENE BIO-TECH PVT. LTD.
GF, Plot no 13, 14, Kamla Amrut Inditech Park, Chhatral- Kadi Road, Indrad, Kadi, Mahesana, Gujarat 382715
Mobile : +91 97 27 37 9000
Email: info@labgene.in
Web: www.labgene.in

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