LIQUIZYME

UREA

(UV GLDH Method)

BEACON

Code	Product Name	Pack Size
LS028A	Liquizyme Urea	50 ml
LS028B	Liquizyme Urea	100 ml
LS028C	Liquizyme Urea	200 ml
LS028D	Liquizyme Urea	500 ml
LS028F	Liquizyme Urea	1000 ml

Intended Use

Diagnostic reagent for quantitative *in vitro* determination of Urea in human serum, plasma and urine.

Clinical Significance

Urea is the major end product of protein nitrogen metabolism in humans. It constitutes the largest fraction of the non-protein nitrogen component of the blood. Urea is produced in the liver and excreted through the kidneys in the urine. Consequently, the circulating levels of urea depend upon protein intake, protein catabolism and kidney function. Elevated urea levels can occur with dietary changes, diseases which impair kidney function, liver diseases, congestive heart failure, diabetes and infections.

Principle

The enzyme methodology employed in this reagent is based on the reaction first described by Talke and Schubert. To shorten and simplify the assay, the calculations are based on the discovery of Tiffany et al. that urea concentration is proportional to absorbance change over a fixed time interval.

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

$$NH_3 + \alpha\text{-KG} + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-Glutamate} + \text{NAD}$$

1. Urea is hydrolysed in the presence of water and Urease to produce ammonia and carbon dioxide.
2. In the presence of GLutamate Dehydrogenase (GLDH) and reduced Nicotinamide Ademine Dinucleotide (NADH), ammonia combines with α-ketoglutarte (α-KG) to produce L-Glutamate.
3. The reaction is monitored by measuring the rate of decrease in absorbance at 340 nm as NADH is converted to NAD.

Reagent Composition

Reagent 1 : Urea Enzyme Reagent

Tris Buffer	:	>100 mmol/L
ADP	:	>1 mmol/L
Urease	:	>20000 U/L
GLDH	:	>1500 U/L
2-Oxalagularate	:	>15 mmol/L

Reagent 2 : Urea Substrate Reagent

NADH : >1.05 mmol/L

Also contains Non-reactive fillers and stabilizers.

Reagent 3 : Urea Standard : 50 mg/dl

Ready to use

Materials Required But Not Provided

- Clean & Dry container.
- Laboratory Glass Pippetes or Micropioettes & Tips.
- Colorimeter or Bio-Chemistry Analyzer.

Stability And Storage

The unopened reagents are stable till the expiry date stated on the bottle and kit label when stored at 2–8°C. Reagents are ready to use. After opening, reagents are stable until expiry date at 2–8°C if stored at appro-priate conditions, closed carefully and without any contamination.

Working Reagent Preparation

Mix 4 portion of reagent R1 with 1 portion of reagent R2.

Stability :

5 days (in the dark)	:	at 15 – 25°C
4 week (in the dark)	:	at 2 – 8°C

Specimen Collection And Handling

Use serum, EDTA plasma and heparin (no ammonium heparin) plasma, urine. It is recommended to follow NCCLS procedures (or similar standardized conditions). Dilute urine 1 + 100 with dist. water and multiply results by 101.

Stability In Serum / Plasma :

7 days	:	at 20 – 25°C
7 days	:	at 4 – 8°C
In Urine :		
1 year	:	at - 20°C
2 days	:	at 20 – 25°C
2 days	:	at 4– 8°C
1 month	:	at - 20°C

Discard contaminated specimens.

Calibration

Calibration with the Urea standard provided in the kit is recommended.

Quality Control

It's recommended to run normal and abnormal control sera to validate reagent performance.

Unit Conversion

mg/dl x 0.1665 = mmol/l

Urea (mg/dl) x 0.467 = BUN (mg/dl)

BUN (mg/dl) x 2.14 = Urea (mg/dl)

Expected Values

In Serum / Plasma	:	10 - 40 mg/dl
Urea in Urine	:	26 – 43 g/24 h (0.43 – 0.72 mol/24 h)

It is recommended that each laboratory verify this range or derives reference interval for the population it serves.

Performance Data

Data contained within this section is representative of performance on Beacon system. Data obtained in your laboratory may differ from these values.

Limit of quantification	:	1 mg/dl
Linearity	:	300 mg/dl
Measuring range	:	1 – 300 mg/dl

Precision

Intra-assay precision Within run (n=20)	**Mean (mg/dl)**	**SD (mg/dl)**	**CV (%)**
Sample 1	42.89	1.06	2.48
Sample 2	111.96	1.62	1.45

Inter-assay precision Run to run (n=20)	**Mean (mg/dl)**	**SD (mg/dl)**	**CV (%)**
Sample 1	24	1.12	4.60

Comparison

A comparison between Beacon Urea (y) and a commercially available test (x) using 20 samples gave following results :

y = 1.055 x - 2.825 mg/dl

r = 0.998

Interferences

Following substances do not interfere :

haemoglobin up to 7.5 g/l, bilirubin up to 30 mg/dl, triglycerides up to 2000 mg/dl.

Warning And Precautions

For *in vitro* diagnostic use. To be handles by entitled and professionally educated person. Reagents of the kit are not classified like dangerous but contains less than 0.1% sodium azide - classified as very toxic and dangerous substance for the environment.

Waste Management

Please refer to local legal requirements.

Assay Procedure

Wavelength	:	340 nm
Cuvette	:	1 cm

Addition Sequence	Standard	Sample
Working Reagent	1000 µl	1000 µl
Standard	10 µl	-
Sample	-	10 µl

Mix and read the initial absorbance A1 for the Standard and Test after exactly 30 seconds. Read another absorbance A2 of the Standard and the test exactly 60 seconds later. Calculate the change in absorbance ΔA for both the Standard and Test.

Calculation

$$\text{Urea (mg/dl)} = \frac{\text{Abs. T}}{\text{Abs. S}} \times 50$$

Applications for automatic analysers are available on request.

Assay Parameters For Photometers

Mode	Fixed time
Wavelength 1 (nm)	340
Sample Volume (µl)	10
Working Reagent Volume (µl)	1000
Lag time (sec.)	30
Read Time (sec.).	60
Reaction temp. (°C)	37
Reaction Direction	Decreasing
Normal Low (mg/dl)	10
Normal High (mg/dl)	40
Linearity Low (mg/dl)	1
Linearity High (mg/dl)	300
Standard Concentration	50 mg/dl
Blank with	Water
Unit	mg/dl

References

1. Shephard, MD, Mezzachi, RD. Clin. Biochem. Revs. 1983; 4: 61-7.

Symbols Used On Labels

REF	Catalogue Number		Manufacturer
	See Instruction for Use	LOT	Lot Number
CONT	Content		Storage Temperature
	Expiry Date	IVD	In Vitro Diagnostics

BEA/24/UUV/LS/IFU-02 22/04/2022