SBio UREA KIT

(GLDH Kinetic Method)

(For invitro diagnostic use only)

REF	90860075	90872150
Pack Size	75 ml	2 x 150 ml

CE

8°C 2°C Store at 2-8°C	Manufacturer	IVD *In vitro* Diagnostic Medical Device	L2 Starter Reagent	GLDH Kinetic GLDH Kinetic Method
Use by (Last day of stated month)	Consult Instructions for use	LOT Batch Number	S Urea Standard (40 mg/dl)	EC REP Authorised Representative in the European Community
Date of Manufacture	REF Catalogue Number	L1 Enzyme Reagent	This way up	

INTENDED USE

Urea Kit is used for the determination of Urea in serum, plasma and urine.

PRINCIPLE OF THE TEST

Urease hydrolyzes urea to ammonia and CO_2. The ammonia formed further combines with α Ketoglutarate and NADH to form Glutamate and NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance in a fixed time, which is proportional to the urea concentration in the sample.

$$\text{Urea} + H_2O + 2H^+ \xrightarrow{\text{Urease}} 2NH_4^+ + CO_2$$

$$2NH_4^+ + 2\alpha\text{ Ketoglutarate} + 2\text{NADH} \xrightarrow{\text{GLDH}} 2\text{ L-glutamate} + 2NAD^+ + 2H_2O$$

CLNICAL SIGNIFICANCE

Urea is the end product of the protein metabolism. It is synthesized in the liver from the ammonia produced by the catabolism of amino acids. It is transported by the blood to the kidneys from where it is excreted. Increased levels are found in renal diseases, urinary obstructions, shock, congestive heart failure and burns. Decreased levels are found in liver failure and pregnancy.

PRESENTATION	75 ml	2 x 150 ml
L1 : Enzyme Reagent	60ml	2 x 120 ml
L2 : Starter Reagent	15 ml	2 x 30 ml
S : Urea Standard (40 mg/dl)	5 ml	5 ml

COMPOSITION

Tris Buffer 100 mM; pH 7.8; Urease > 7500 U/L; GLDH > 1000 U/L; NADH 0.18 mM; Ketoglutarate 7 mM; Non-Reactive Stabilizers, Detergent and Preservative.

STORAGE / STABILITY

Contents are stable at 2-8°C till the expiry mentioned on the labels.

SAMPLE REQUIRED

Serum, plasma, Urine. Dilute urine specimen 1+ 49 with distilled water before the assay (results x 50). Urea is reported to be stable in serum for 5 days at 2-8°C.

REAGENT PREPARATION

Reagents are ready to use.

Working reagent: For sample start assays a single reagent is required. Pour the contents of 1 bottle of L2 (Starter Reagent) into 1 bottle of L1 (Enzyme Reagent). This working reagent is stable for at least 10 days when stored at 2-8°C.

Alternatively for flexibility as much of working reagent may be made as and when desired by mixing together 4 parts of L1 (Enzyme Reagent) and 1 part of L2 (Starter Reagent). Alternatively 0.8 ml of L1 and 0.2 ml of L2 may also be used instead of 1 ml of the working reagent directly during the assay.

Allow the working reagent to stand for 30 min. before use.

SAMPLE WASTE AND DISPOSAL

Do not reuse the reagent containers, bottles, caps or plugs due to the risks of contamination and the potential to compromise reagent performance.

This product requires the handling of human specimen. It is recommended that all human sourced material are considered potentially hazardous and are handled in accordance with the OSHA standard on blood borne pathogens.

Appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.

Handle specimen, solid and liquid waste and test components in accordance with local regulations and NCCLS guidelines M29, or other published biohazard safety guidelines.

PROCEDURE

Wavelength/filter	:	340 nm
Temperature	:	37° C / 30° C / 25° C
Light path	:	1 cm

MATERIALS REQUIRED BUT NOT PROVIDED

General laboratory instrumentation like Spectrophotometer/Analyzer, Thermostatic Cuvette holder, Cuvettes, Micropipettes, Test tubes, Waterbath, Stopwatch/Timer.

Substrate Start Assay:

Pipette into clean dry test tubes labeled as Standard (S) or Test (T):

Addition Sequence	(S) / (T) 37° C / 30° C / 25° C
Enzyme Reagent (L1)	0.8 ml
Urea Standard / Serum / Diluted Urine	0.01 ml
Incubate at the assay temperature for 1 minute and add	
Starter Regent (L2)	0.2 ml

Mix well and read the initial absorbance A_1 for the Standard and Test after exactly 30 seconds. Read another absorbance A_2 of the Standard and Test exactly 60 seconds later. Calculate the change in absorbance ΔA for both the Standard and Test.

Sample Start Assay:

Pipette into clean dry test tubes labeled as Standard (S) or Test (T):

Addition Sequence	(S) / (T) 37° C / 30° C / 25° C
Working Reagent	1.0 ml
Bring to assay temperature and add	
Urea Standard / Serum / Diluted Urine	0.01 ml

Mix well and read the initial absorbance A_1 for the Standard and Test after exactly 30 seconds. Read another absorbance A_2 of the Standard and Test exactly 60 seconds later. Calculate the change in absorbance ΔA for both the Standard and Test.

For Standard $\Delta AS = A_2S - A_1S$

For Test $\Delta AT = A_2T - A_1T$

CALCULATIONS

$$\text{Urea in mg/dl} = \frac{\Delta AT}{\Delta AS} \times 40$$

QUALITY CONTROL

The following process is recommended for QC during the assay of Urea.

*Define and establish acceptable range for your laboratory.

1. Two levels of control (Normal and Abnormal) are to be run on a daily basis.
2. If QC results fall outside acceptance criteria, recalibration may be necessary.
3. Review QC results and run acceptance criteria following a change of reagent lot.

SPECIFIC PERFORMANCE CHARACTERISTICS

Linearity:

The procedure is linear upto 250 mg/dl. If values exceed this limit, dilute the serum with normal saline (NaCl 0.9%) and repeat the assay. Calculate the value using the proper dilution factor.

Limit of detection:

The limit of detection for Urea is 1 mg/dl.

Interferences:

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Precision:

Precision studies were performed with two controls using NCCLS protocol EP5-A. The results of the precision studies are shown below:

Sample	Within-run		Between-run		Total	
	Mean	CV%	Mean	CV%	Mean	CV%
Control 1	39.88	3.56	40.37	2.74	80.25	6.30
Control 2	153.40	4.59	161.08	1.50	314.48	6.09

Method comparison:

Comparative studies were done to compare our reagent with another commercial Urea Assay. No significant differences were observed. Details of the comparative studies are available on request.

REFERENCE RANGE

Serum / Plasma	: 14 - 40 mg/dl
Urine	: Upto 20 g/l

It is recommended that each laboratory establish its own normal range representing its patient population*.

NOTE

Plasma should not be collected with Fluoride or Heparin salts as contamination by ammonia or ammonium salts lead to erroneous results. The reagent may be used in several automated analyzers. Instructions are available on request.

Standard is traceable to standard reference material (SRM) 909b. Do not use turbid, deteriorated or leaking reagents.

REFERENCES

1. Fawcett J.K. Scott J.E. (1960) J. Chim. Pathol. 13 : 156.
2. Chaey A., (1962) Clin. Chem. 8 : 130.

Mfd. for:

Singapore SB Biosciences PTE Ltd.

11 Yishun Street 51, # 04-23, The Criterion, Singapore 767971.

EC REP

CMC Medical Devices & Drugs S.L.,
C/ Horacio Lengo No. 18, CP 29006, Malaga, Spain.

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