SBio UREA KIT

(Mod. Berthelot Method)

(For invitro diagnostic use only)

REF	90820075
Σ	75 Assays

CE

8°C 2°C Store at 2-8°C	Manufacturer / This way up	IVD *In vitro* Diagnostic Medical Device	L1 Buffer Reagent	MOD. BERTHELOT Mod. Berthelot Method	
Use by (Last day of stated month)	Consult Instructions for use	LOT Batch Number	L2 Enzyme Reagent	S Urea Standard (40 mg/dl)	
Date of Manufacture	REF Catalogue Number	Σ Contains sufficient for <n> tests	L3 Chromogen Reagent	EC REP Authorised Representative in the European Community	

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 reacts with a phenolic chromogen and hypochlorite to form a green coloured complex. Intensity of the colour formed is directly proportional to the amount of urea present in the sample.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} \text{Ammonia} + CO_2$$

$$\text{Ammonia} + \text{Phenolic chromogen} + \text{Hypochlorite} \longrightarrow \text{Green coloured complex}$$

CLINICAL SIGNIFICANCE

Urea is the major metabolic product of nitrogen substances in the body. Urea is found in body fluids and tissues in approximately equal concentrations. The concentration of urea in whole blood is lower, as erythrocytes contain less urea. Urea is excreted in urine. 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 Assays
L1 : Buffer Reagent	75 ml
L2 : Enzyme Reagent	7.5 ml
L3 : Chromogen Reagent	15 ml
S : Urea Standard (40 mg/dl)	5 ml

COMPOSITION

Phosphate Buffer 50mmol; Nitroprusside > 10mmol; Phenolic Chromogen > 40.0mmol; Hypochlorite > 500mmol; Non Reactive Stablizers; Detergents and preservatives.

STORAGE / STABILITY

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

REAGENT PREPARATION

Reagents are ready to use.

Working Enzyme Reagent: For the flexibility and convenience in performing large assay series, a working enzyme reagent may be made by pouring 1 bottle of L2 (Enzyme Reagent) into 1 bottle of L1 (Buffer Reagent). For smaller series combine 10 parts of L1 (Buffer Reagent) and 1 part of of L2 (Enzyme Reagent). Use 1ml of the working reagent per assay instead of 1 ml of L1 and 0.1 ml of L2 as given in the procedure. The working enzyme reagent is stable for at least 4 weeks when stored at 2-8°C.

Working Chromogen Reagent: For larger volume cuvettes, dilute 1 part of L3 (Chromogen Reagent) with 4 parts of fresh ammonia free distilled/deionised water. Use 1ml of working chromogen instead of 0.2 ml in the assay. The working chromogen reagent is stable for at least 8 weeks when stored at 2-8°C in a tightly stoppered plastic bottle.

SAMPLE REQUIRED

Serum, plasma or urine is required. Dilute urine 1+ 49 with distilled /deionised water before the assay (Results x 50).

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 : 570 nm (Hg 578 nm) / Yellow
Temperature : 37° C / R.T.
Light path : 1 cm

MATERIAL REQUIRED BUT NOT PROVIDED

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

Pipette into clean dry test tubes labeled as Blank (B), Standard (S) and Test (T):

Addition Sequence	B (ml)	S (ml)	T (ml)
Buffer Reagent (L1)	1.0	1.0	1.0
Enzyme Reagent (L2)	0.1	0.1	0.1
Distilled Water	0.01	--	--
Urea Standard (S)	--	0.01	--
Sample	--	--	0.01
Mix well and incubate for 5 mins. at 37° C or 10 mins. at R.T. (25° C)			
Chromogen Reagent (L3)	0.2	0.2	0.2

Mix well and incubate at 37°C for 5 minutes or at R.T. (25° C) for 10 minutes. Measure the absorbance of the Standard (Abs. S) and Test Sample (Abs. T) against the Blank within 60 minutes.

CALCULATIONS

$$\text{Urea in mg/dl} = \frac{\text{Abs. T}}{\text{Abs. S}} \times 40$$

$$\text{Urea Nitrogen in mg/dl} = \text{Urea in mg/dl} \times 0.467$$

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. Using the working chromogen reagent (1ml) the linearity is increased to 400 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.

Method comparison:

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

Urea is reported to be stable in the serum for 5 days when stored at 2-8° C. Any contamination by ammonia or ammonium salts lead to erroneous results, hence plasma should not be collected with Fluoride or Heparin Ammonium salts. The working enzyme reagent is not stable at elevated temperatures and should be stored back at 2-8°C immediately after use. The chromogen reagent contains chlorine. The bottles should be opened only when required and closed tightly after use to prevent the loss of active chlorine. Do not use turbid, deteriorated or leaking reagents.

REFERENCES

(1) Berthelot, M.P.E., (1859) Report Chim. Appl. 2884.
(2) Fawcett J.K. Scott J.E. (1960) J. Chim. Pathol. 13 : 156.

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EC REP

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