NEO CHEM
UREA (E.P)

(Mod. Berthelot method)

KIT NAME	KIT SIZE
NEO CHEM - Urea (E.P)	2 X 50 ML

NEOGENIX
DIAGNOSTICS PVT LTD

Intended Use:
Urea test reagent/kits is a medical device intended for the estimation of urea/Blood in serum or plasma.

Clinical Significance
Urea is a nitrogen-containing end product of protein catabolism. States associated with elevated levels of urea in blood are referred to as hyper uremia or azotemia. Parallel determination of urea and creatinine is performed to differentiate between pre-renal and post-renal azotemia. Pre-renal azotemia, caused by e.g. dehydration, increased protein catabolism, cortisol treatment or decreased renal perfusion, leads to increased urea levels, while creatinine values remain within the reference range. In post-renal azotemias, caused by the obstruction of the urinary tract, both urea and creatinine levels rise, but creatinine in a smaller extent. In renal diseases urea concentrations are elevated when the glomerular filtration rate is markedly reduce and when the protein intake is higher than 200g/day.

Principle
Urea is converted quantitatively by urease into ammonia and carbon di oxide. In this modified method ammonium ions reacts with hypochlorite and salicylate to give a green colored complex (Berthelot reaction). The color yield is enhanced by Sodium nitroprusside. The color intensity is directly related to the urea concentration and is measured photometrically at 578 nm.

Reagent Composition:

R1 Buffer	Sodium Salicylate - 40 mmol/L Activator & Stabilizers
R2 Enzyme Reagent	Urease – 30,000 U/L
R3 Chromogen	Sodium Hypochlorite - 10 mmol/L Sodium Hydroxide - 400 mmol/L
Standard	40mg/dl

Working Reagent Preparation
Reagent is ready to use.
Working enzyme reagent may be made by pouring 1 bottle R2 Enzyme reagent into 1 bottle of R1 Buffer reagent.
Working reagent is stable for 16 weeks when proper Storage conditions are strictly maintained.

Stability and Storage
Store at 2-8ºC
All the kit contents are stable until the expiry date stated on thelabel. Do not use reagents beyond the expiration date.
Store the vials tightly closed protected from light and preventcontaminations during the use.

Discard if signs of deterioration appear:
- Presence of particles and turbidity.

Materials required
- Photometer or spectrophotometer with a thermostat cellcompartment set at 25/30/37ºC, capable of reading at 578 (570-620) nm.
- Stopwatch, strip-chart recorder or printer.
- Cuvettes with 1-cm path length
- Pipettes to measure reagent and samples.

Sample and Stability
Serum or plasma
Serum or heparinized plasma free of hemolysis.
Urea is reported to be stable in the sample for 7days when stored at 2-8^{0}C.

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

Addition Sequence	**Blank**	**Std**	**Test**
Working Reagent (R1+R2)	1000µl	1000µl	1000µl
Standard (S)	-	10µl	-
Sample	-	-	10µl
Mix, incubate for 3 min. at 37 °C or5 Min at RT and the add			
R3 Chromogen Reagent	1000µl	1000µl	1000µl

Mix well and incubate at 37°C for 5 min or at R.T. for 10 min. Measure the absorbance of the standard (Absorbance of Standard) and test Sample(Absorbance of Test) against reagent blank at 578(570 – 620 nm). Final colour is stable 10 hours at R.T (<30°C)

Calculation:

$$\text{Urea in mg/dl} = \frac{\text{Abs of Test}}{\text{Abs of Std}} \times 40$$

Quality Control
To ensure adequate quality control (QC), each run should include aset of controls (normal and abnormal) with assayed values handledas unknowns.
If the values are found outside of the defined range, check the instrument, reagents and procedure.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable results

Linearity
This procedure is linear upto 350 mg/dl. If values exceed this limit, dilute the sample with normal saline(NaCl 0.9%) and repeat assay.Calculate the value using the proper dilution factor.

Reference Values
Serum and Plasma : 14 - 40 mg/dl
Urine : 20 g/L
It is recommended that each laboratory establishes its own normal range representing its patient population.

General System Parameters

Mode	End Point
Reaction	Increasing
Wavelength	578 (570-620) nm
Blank with	Reagent
Sample Volume	10µl
Reagent Volume	1000µl+ 1000µl
Std Conc.	40 mg/dl
Incub.Temp.	37^{0}C
Incub. Time	3min + 5min
Delay Time	5 sec
Normal Range	14 – 40 mg/dl
Linearity	350mg/dl
Unit	mg/dl

Notes

1. Enzyme Reagent (R2) may appear slightly hazy, but after mixing it with Buffer Reagent (R1) its haziness disappears and this does not affect the performance of kit

2. Any contamination by ammonia or ammonium salts lead to erroneous results. Hence plasma should not be collected within fluoride or Heparin ammonium salts.

3. The working enzyme reagent is not stable at elevated temperatures and should be stored back at 2-8°C immediately after use.

4. The chromogen reagent contains chlorine. Reagent bottles should be opened only when required and closed tightly after use to prevent the loss of active chlorine.

Reference

1.Berthelot,M.P.E,(1859)Report chim.Appl.2884

2.Fawcett,j.k.Scott,J.E.,(1960) J.Chim. Pathol .13:156

For *in vitro* Diagnostic use only.