Cromatest

LINEAR

UREA/BUN BR CE

REF 1158005	REF 1158010
2 x 50 mL	3 x 100 mL
CONTENTS	**CONTENTS**
R1. Reagent 2 x 40 mL	R1. Reagent 3 x 80 mL
R2. Reagent 1 x 20 mL	R2. Reagent 1 x 60 mL
CAL. Standard 1 x 3 mL	CAL. Standard 1 x 3 mL
For *in vitro* diagnostic use only	

UREA/BUN BR

Urease/GlDH

UV enzymatic method

KINETIC

PRINCIPLE

Urea is hydrolyzed by urease to ammonia and carbon dioxide. The ammonia is converted to glutamate by glutamate dehydrogenase (GlDH) in the presence of NADH and oxoglutarate.[1,2]
The reaction is monitored kinetically at 340 nm by the rate of decrease in absórbanse resulting from the oxidation of NADH to NAD^+, proportional to the concentration of urea present in the sample.

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

$$\text{2-Oxoglutarate} + NH_4^+ + 2\ \text{NADH} \xrightarrow{\text{GlDH}} \text{Glutamate} + 2\ NAD^+ + 2\ H_2O$$

REAGENT COMPOSITION

R1 **Buffered Urease/GlDH**. TRIS buffer 125 mmol/L pH 7.4, 2-oxoglutarate 10 mmol/L, urease > 140 U/mL, glutamate dehydrogenase > 120 U/mL, Biocides.

R2 **Coenzyme**. NADH 1.50 mmol/L.

CAL **Urea standard.** Urea 50 mg/dL (8.3 mmol/L).
Organic matrix based primary standard. Concentration value is traceable to Standard Reference Material 909b.

STORAGE AND STABILITY

Store at 2-8ºC.
All the kit compounds are stable until the expiry date stated on the label. Do not use reagents over the expiration date.
Store the vials tightly closed, protected from light and prevented contaminations during the use.

Discard If appear signs of deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 340 nm < 1.100 in 1cm cuvette.

REAGENT PREPARATION

Working reagent. Mix 4 mL of **R1** + 1 mL of **R2**. Stable for 2 months at 2-8ºC.

SAMPLES

Serum or heparinized plasma free of hemolysis and urine (see Notes). Other anticoagulants (ammonium heparinate or double oxalate of potassium and ammonium) must not be used.
Urea in serum, plasma or urine is stable 7 days at 2-8ºC.
Freeze for longer storage.

INTERFERENCES

- Lipemia (intralipid < 5 g/L) does not interfere.
- Bilirubin (40 mg/dL), hemoglobin (< 4 g/L), do not interfere.
- Other drugs and substances may interfere[3].
- Contamination of glassware and water by ammonia, will give falsely elevated results.
- Fluorides used commonly as anticoagulants inhibit the urease of the substrate[4].

MATERIALS REQUIRED

- Photometer or spectrophotometer with a thermostatted cell compartment set at 37ºC, capable of reading at 340 nm.
- Stopwatch, strip-chart recorder or printer.
- Cuvettes with 1-cm pathlength.
- Pipettes to measure reagent and samples.

PROCEDURE

1. Preincubate working reagent, samples and standard to reaction temperature.
2. Set the photometer to 0 absorbance with distilled water.
3. Pipette into a cuvette:

Reaction temperature	37ºC
Working reagent	1.0 mL
Sample or standard	10 µL

4. Mix gently by inversion. Insert cuvette into the cell holder and start stopwatch.
5. Record the absorbance at 340 nm exactly after 30 seconds (A_1) and exactly 90 seconds later (A_2).
6. Calculate the difference between absorbances.

CALCULATIONS

Serum, plasma

$$\frac{(A_1 - A_2)_{\text{Sample}}}{(A_1 - A_2)_{\text{Standard}}} \times C_{\text{Standard}} = \text{mg/dL urea}$$

Samples with concentrations higher than 500 mg/dL should be diluted 1:2 with saline and assayed again. Multiply the results by 2.

Urine

Dilute the sample 1:50 with distilled water and multiply the result by 50.

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If results are to be expressed as SI units apply:
mg/dL x 0.1665 = mmol/L

To convert urea mass units to those of urea nitrogen apply:
mg/dL x 0.467 = mg/dL BUN

REFERENCE VALUES [5]

Serum, plasma

Newborns (< 10 days)	6.4 - 53.5 mg/dL (1.1 - 9.0 mmol/L)
Adults (12-60 years)	15 - 40 mg/dL (2.5 - 6.6 mmol/L)

In adults over 60 years of age, the reference interval is 17-50 mg/dL (2.8-8.3 mmol/L) and the concentrations tend to be slightly higher in males than in females.

Urine

Adults (normal diet)	26 - 43 g/24-h (428 - 714 mmol/24-h)

A high-protein diet causes significant increases in plasma urea concentrations and urinary excretion.
It is recommended that each laboratory establishes its own reference range.

QUALITY CONTROL

The use of a standard to calculate results allows to obtain an accuracy independent of the system or instrument used.
To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.

REF **1980005** HUMAN MULTISERA NORMAL
Borderline level of urea. Assayed.

REF **1985005** HUMAN MULTISERA ABNORMAL
Elevated level of urea. Assayed.

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 tolerances.

CLINICAL SIGNIFICANCE

Urea is the chief end product of protein metabolism in the body. The importance of the urea concentration in blood lies in its value as an indicator of kidney function.

Azotemia (an abnormal increase in plasma urea level) is seen mainly in renal disorders, dehydration, increase protein catabolism, high-protein diets, or gastrointestinal hemorrhage. There are two types of azotemia. The first, *prerenal azotemia*, is caused by impaired perfusion of the Kidneys due to decreased cardiac output or for any of the former causes. The second, *postrenal azotemia*, is caused by an obstruction in the urine outflow such as nephrolithiasis, prostatism, and tumors of the genitourinary tract.
The clinical significance of the urea level in plasma is usually determined in conjugation with the plasma creatinine level. In prerenal azotemia, an increase in the plasma urea level is usually associated with a normal plasma creatinine level, where as in postrenal azotemia, there is an increase in both the urea and the plasma creatinine levels. A decrease in the urea plasma level may be associated with acute dehydration, malnutrition, and pregnancy.

NOTES

- Collect a 24-hour urine specimen into a plastic bottle free of preservatives. Keep the sample refrigerated to minimize urea hydrolysis by microorganisms or other agents.
- This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meet the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact to the distributor for any question on the application method.
- Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

ANALYTICAL PERFORMANCE

- ***Detection Limit :*** 1.14 mg/dL
- ***Linearity :*** Up to 500 mg/dL
- ***Precision:***

mg/dL	Within-run		Between-run	
Mean	47.8	121.6	47.8	121.6
SD	0.43	0.87	0.82	2.25
CV%	0.91	0.71	1.72	1.85
N	10	10	10	10

- ***Sensitivity :*** Δ 3 mA/min / mg/dL Urea.
- ***Correlation.*** This assay (y) was compared with a similar commercial method (x). The results were:
  N = 57 r = 1.00 y = 1.03x + 0.86

The analytical performances have been generated using on automatic instrument. Results may vary depending on the instrument.

REFERENCES

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3. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
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5. Tietz. N.W. Clinical Guide to Laboratory Tests, 3rd Edition. W.B. Saunders Co. Philadelphia, PA. (1995).

QUALITY SYSTEM CERTIFIED
ISO 9001 ISO 13485

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