UREA U.V.

Enzymatic method for the quantitative determination of Urea in serum, plasma and urine

ORDER INFORMATION

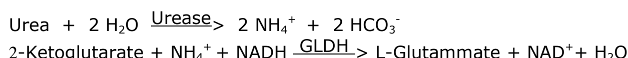
REF	Kit size
GA4960 00	10x40 + 5x20 ml
KL4960 00	10x40 + 10x10 ml
BK4960 00	5x(60+15 ml)

INDICATION

Urea concentration is an indicator of kidney function. Conditions associated to high urea values are related to iperuremia and azotemia.

METHOD PRINCIPLE

Urease hydrolyzes urea into ammonia and carbon dioxide. Glutamate dehydrogenase catalyzes the reaction of ammonia with 2-ketoglutarate and oxidizes NADH into NAD^+.



Urea + 2 H_2O —Urease→ 2 NH_4^+ + 2 HCO_3^-
2-Ketoglutarate + NH_4^+ + NADH —GLDH→ L-Glutammate + NAD^+ + H_2O

The decrease of absorbance of NADH, measured at 340 nm, is proportional to the urea present in the sample.

COMPOSITION

REAGENT A:

TRIS pH 7.8	150 mmol/l
2-Ketoglutarate	8.75 mmol/l
ADP	0.75 mmol/l
Urease	≥ 7.5 kU/l
GLDH (Glutamate-dehydrogenase)	≥ 1.25 kU/l
Sodium azide	≤ 0.95 g/l

REAGENT B:

NADH	1.32 mmol/l
Sodium azide	≤ 0.95 g/l

STANDARD: 1x5 ml
Urea 50 mg/dl
Verified against NIST reference material.

PREPARATION OF REAGENTS

Bireagent procedure:
The reagents are liquids ready to use.

Monoreagent procedure:
Mix 4 parts of Reagent A and 1 part of Reagent B to obtain the working reagent (e.g. 20 ml of RA + 5 ml of RB).
Let stand working reagent at least 30 minutes at room temperature before use.

Storage and stability
Store at 2-8 °C. Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label, if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature.

Working reagent is stable for 28 days at 2-8 °C or 5 days at 15-25 °C, protected from light.

ANCILLARY EQUIPMENT

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l

SAMPLES

Serum, plasma, 24h urine.
Do not use anticoagulants containing fluoride or ammonium ions.
Dilute urine 1:20 with distilled water.

Stability:	Temperature		
	20-25 °C	4-8 °C	- 20 °C
Serum/plasma:	7 days	7 days	1 year
Urine:	2 days	7 days	1 month

Specimen collection / Preanalytical factors
It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

INTERNAL QUALITY CONTROL

It is recommended to use commercial Quality Control sera with known urea concentration. Check that the values obtained are within the reference range provided.

ANALYTICAL PROCEDURE

Working temperature	37 °C
Wavelength	340 nm (334 nm, 365 nm)
Optical path	1 cm
Reaction	fixed time (decrease)

Allow the reagents to reach working temperature before using.

Bireagent procedure

Pipette into disposable or well clean cuvettes:

	Blank	Standard	Sample
Reagent A	800 μl	800 μl	800 μl
Standard	-	10 μl	-
Sample	-	-	10 μl
Mix and incubate for 5 minutes at 37 °C. Then add:			
Reagent B	200 μl	200 μl	200 μl
Mix, incubate for 30 seconds at 37 °C, than read A_1 of sample, standard and Blank. After precisely 60 seconds read absorbance A_2. Determine: $\Delta A = [(A_1 - A_2)$ sample or standard$]-[(A_1 - A_2)$ Blank$]$			

Monoreagent procedure

Pipette into disposable or well clean cuvettes:

	Bianco	Standard	Campione
Working reagent	1000 μl	1000 μl	1000 μl
Standard	-	10 μl	-
Sample	-	-	10 μl
Mix, incubate for 30 seconds at 37 °C, than read A_1 of sample, standard and Blank. After precisely 60 seconds read absorbance A_2. Determine: $\Delta A = [(A_1 - A_2)$ sample or standard$]-[(A_1 - A_2)$ Blank$]$			

Note

- Reaction volumes can be proportionally changed.
- The method is optimized for "two points" determinations. It is absolutely necessary to incubate reagent blank, standard and samples exatly for the same time. The same preincubation time for reagent blank, standard and sample is also necessary.

CALCULATION OF RESULTS

Serum-plasma:

$$\text{Urea, mg/dl} = \frac{\Delta A\ \text{sample}}{\Delta A\ \text{standard}} \times 50$$

Urine (when 24h diuresis is known):

$$\text{Urea, g/24h} = \frac{\Delta A\ \text{sample}}{\Delta A\ \text{standard}} \times 10 \times \text{l/24h}$$

Conversion factor
Urea [mg/dl] x 0.1665 = Urea [mmol/l]
Urea [mg/dl] x 0.467 = BUN* [mg/dl]

* Blood Urea Nitrogen

REFERENCE VALUES

Serum-plasma: 18÷53 mg/dl (adults)
Urine 24h: 6÷17 mg/24h

Each laboratory should establish reference ranges for its own patients population.

ANALYTICAL PERFORMANCES

Precision
Within-run and between-run coefficients of variation have been calculated on replicates of two sera with different urea concentration. The obtained results are reported in the following table:

		Within-run		Between-run	
Sample	**Mean (mg/dl)**	**SD**	**%CV**	**SD**	**%CV**
Siero 1	42.8	1.54	3.6	1.50	3.5
Siero 2	161.7	3.60	2.2	7.51	4.6

Linearity
The assay is linear up to 300 mg/dl.

Sensitivity
Test sensitivity, in terms of limit of detection, is 2 mg/dl.

Correlation
A correlation study comparing the present method and a commercial one gave the following results:

y = 1.0436x - 1.1064 mg/dl r = 0.9924

Interferences
Hemoglobin > 500 mg/dl
Bilirubin > 40 mg/dl
Triglycerides > 2000 mg/dl
Ascorbic acid > 30 mg/dl

PRECAUTIONS IN USE

The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes. The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management
Please refer to local legal requirements.

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