

UREA UV KIT

Urease-GLDH. Kinetic. Liquid

For *in-vitro* diagnostic use only.

Store at 2-8°C.

PRINCIPLE OF THE METHOD

Urea in the sample is hydrolyzed enzymatically into ammonia (NH₄⁺) and carbon dioxide (CO₂).

Ammonia ions formed reacts with α-ketoglutarate in a reaction catalysed

by glutamate dehydrogenase (GLDH) with simultaneous oxidation of

NADH to NAD⁺:



The decrease in concentration of NADH, is proportional to urea concentration in the sample¹

CLINICAL SIGNIFICANCE

Urea is the final result of the metabolism of proteins; It is formed in the liver from their destruction

It can appear the urea elevated in blood (uremia) in: diets with excess of proteins, renal diseases, heart failure, gastrointestinal hemorrhage, dehydration or renal obstruction.

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R 1 Buffer	TRIS pH 7.8 α-Ketoglutarate Urease	80mmol/L 6 mmol/L 75000U/L
R 2 Enzymes	GLDH NADH	60000U/L 0.32mmol/L
UREA STD	Urea aqueous primary standard 50mg/dL	

PREPARATION

Working reagent (WR):

Mix

4 vol. R1 Buffer + 1 vol. R2 Substrate.

The (WR) is stably for 1 month at 2-8°C.

UREA STD: Ready to use.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use.

Do not use reagents over the expiration date.

UREA CAL Once open is stable up to 1 month when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use.

Signs of reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 340 nm < 1.00.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 340 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

- Serum or heparinized plasma: Do not use ammonium salts or fluoride as anticoagulants.

Urine¹: Dilute sample 1/50 in distilled water. Mix. Multiply the results by 50 (dilution factor). Preserve urine samples at pH < 4

Urea is stable at 2-8°C for 5 days

PROCEDURE

1. Assay conditions:

Wavelength.....340 nm

Cuvette:1 cm light path

Temperature:.....37°C / 15-25°C

2. Adjust the instrument to zero with distilled water.

3. Pipette into a cuvette:

	Blank	Standard	Sample
WR (mL)	1.0	1.0	1.0
Standard (μL)	--	10	--
Sample (μL)	--	--	10

4. Mix and Read the absorbance after 30 s (A1) and 90 s (A2)

5. Calculate: ΔA= A1-A2

CALCULATIONS

$$\frac{(A)\text{Sample}}{(A)\text{Standard}} \times 50 (\text{Standard conc.}) = \text{mg/dL}$$

urea in the sample

$$10 \text{ mg/L urea BUN divided by } 0.466 = 21 \text{ mg/L urea} = 0.36 \text{ mmol/L}$$

Conversion factor: mg/dL x 0.1665 mmol/L.

QUALITY CONTROL

If control values are found outside the defined range, check the instrument, reagent and calibration for problems

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES

Serum or plasma:

$$15-45 \text{ mg/dL} = 2.5-7.5 \text{ mmol/L}$$

Urine:

$$26 - 43 \text{ g/24 h} = 428-714 \text{ mmol/24 h}$$

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From detection limit of 1mg/dL to linearity limit of 350 mg/dL.

If the concentration is greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/L)	40.6	141	42.5	141
SD	1.22	1.03	2.12	1.15
CV (%)	2.99	0.73	4.99	0.81

Sensitivity: 1 mg/dL = 0.00087 A.

Accuracy: Results obtained using ATLAS reagents (y) did not show systematic differences when compared with other commercial reagents (x). The results obtained using 50 samples were the following:

Correlation coefficient (r): 0.99.

Regression equation: y=0.9993x + 0.0394.

The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

It is recommended to use heparin as anticoagulant. Do not use ammonium salts or fluoride.

A list of drugs and other interfering substances with urea determination has been reported by Young et. al

NOTES

1. Glassware and distilled water must be free of ammonia and ammonium salts 1.
2. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
3. Use clean disposable pipette tips for its dispensation.

BIBLIOGRAPHY

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