UREA UV

Kinetic UV method - Liquid reagents

R1: 4 x 40 ml + R2: 4 x 10 ml CL54-200

INTENDED USE

Kit for quantitative determination of Urea in serum and plasma. Urease-GLDH method.

PRINCIPLE

In the presence of urease, urea is hydrolyzed to ammonium ion and carbon dioxide. In the presence of glutamate-dehydrogenase (GLDH), the formed ammonium ion reacts with α-ketoglutarate and NADH to form glutamate and NAD^+. Measured at 340 nm, NADH oxidation in unit time is proportional to the urea concentration in the sample.

CLINICAL MEANING

Urea derives from protein catabolism, more than 90% is excreted through the kidney. The increase in plasma urea can occur from kidney or heart failure, loss of water and salts, obstruction of the urinary tract, or increased protein breakdown. A decrease can be found in cases of hyperhydration, severe hepatic insufficiency, increased protein synthesis, deficient protein intake in the diet.

SAMPLE

Serum, heparinized plasma.
Avoid anticoagulants containing ammonium salts and fluoride.
Stability: 3 days at 2-8°C.

REAGENTS

Only for in Vitro diagnostics. Liquid reagents ready to use.
Dangerous reagents are marked by an asterisk. Refer to safety data sheet

Pack Contents	**CL54-200**
REAGENT 1 Tris buffer (pH 7,6) 100 mmol/L, ADP 0,7 mmol/L, α-ketoglutarate 9 mmol/L, urease ≥ 6500 U/L, GLDH ≥ 1100 U/L, sodium azide 15 mmol/L	**CL54-200R1** 4 x 40 ml
***REAGENT 2** Tris buffer (pH 10.2) 10 mmol/L, NADH 1,6 mmol/L, sodium azide 15 mmol/L	**CL54-200R2** 4 x 10 ml
STANDARD (Std) Urea 40 mg/dl (6,65 mmol/L), benzoic acid 15 mmol/L	**CL54-2004** 4 ml

Stability: reagents are ready to use. Store at 2-8°C and protect from light to keep the reagents stable up to the expiration date on the label. Do not freeze. Keep bottles closed when not in use to avoid oxidation and evaporation. Do not use turbid reagents.

PREPARATION OF WORKING SOLUTION
(only for monoreagent procedure)

Mix 4 volumes of Reagent 1 with 1 volume of Reagent 2. Let the reagent stabilize for 20-30 minutes before use.
Stability: 5 days at 20-25°C, 4 weeks at 2-8°C if stored in a closed bottle protected from light.

MANUAL ASSAY PROCEDURE

Analysis:	decreasing kinetic
Wavelength:	340 nm (334 - 365)
Cuvette:	1 cm optical path
Temperature:	37°C
Rate time:	1 minute
Reading:	against air or distilled water
Sample/Reagents (bi):	1/100/25
Sample/Reagent (mono):	1/100

Bireagent procedure

Let the reagent required to perform the test reach the chosen temperature for the analysis. Pipette in cuvette:

	Reagent Blank	Standard	Sample
Distilled water	10 µl	-	-
Standard	-	10 µl	-
Sample	-	-	10 µl
Reagent 1	1,0 ml	1,0 ml	1,0 ml

Mix and incubate 1 minute at 37°C. Add:

Reagent 2	0,25 ml	0,25 ml	0,25 ml

Mix and pour into the test cuvette. Incubate at the test temperature for 30 seconds. Read the initial absorbance, repeat the reading after 60 seconds. Calculate the variation value of the absorbance of the Blank Sample (ΔABR), the Sample (ΔAS) and the Standard (ΔAStd).

Reaction volumes can be proportionally varied without any change in calculation.

Monoreagent procedure

Let the working reagent required to perform the test reach the chosen temperature for the analysis.

Pipette in cuvette:

	Standard	Sample
Standard	10 µl	-
Sample	-	10 µl
Working reagent	1,0 ml	1,0 ml

Mix and pour in the test cuvette. Incubate at the test temperature for 30 seconds. Read the initial absorbance and repeat the reading after exactly 60 seconds. Calculate the variation value of the absorbance of the Sample (ΔAS) and the Standard (ΔAStd).

Reaction volumes can be proportionally varied without any change in calculation.

CALCULATION

Bireagent procedure: calculate the concentration in the sample using the following formula:

Urea [mg/dl] = (ΔAS - ΔABR / ΔAStd- ΔABR) x 40

Urea [mmol/L] = (ΔAS - ΔABR / ΔAStd- ΔABR) x 6,65

Monoreagent procedure: calculate the concentration in the sample using the following formula:

Urea [mg/dl] = (ΔAS / ΔAStd) x 40

Urea [mmol/L] = (ΔAS / ΔAStd) x 6,65

REFERENE VALUES

Serum / plasma: 10 ÷ 50 mg/dl (1,66 ÷ 8,31 mmol/L)

Each laboratory should define its own reference values for this method.

QUALITY CONTROL

A quality control program is recommended for all clinical laboratories.
Control serums of human origin are available for this purpose on request:
PRE-NORM serums with normal values
PRE-PATH serums with pathological values
If the method requires it, a multi-parameter calibrator of human origin is available.

PERFORMANCE CHARACTERISTICS

Sensitivity: the sensitivity of the method is 3 mg/dl.

Linearity: up to 300 mg/dl.
For higher values, dilute the samples 1:10 with saline solution and multiply the result by 10.

Within-run precision:

	Level 1	Level 2
Average [mg/dl]	43,4	138,8
DS	1,12	6,50
CV %	2,6	4,7

Between-run precision:

	Level 1	Level 2
Average [mg/dl]	42,4	137,9
DS	1,33	4,00
CV %	3,14	2,90

Interferences: up to 30 mg/dl of bilirubin does not interfere. Up to 30 mg/dl of ascorbic acid does not interfere. Up to 500 mg/dl of hemolysis does not interfere.

Correlation: the correlation of the method (Y) against a reference method (X) gives this equation:

Y = 1,0126X + 1,218 r = 0,9998

DISPOSAL

The product must be used for professional analysis only. The product must be disposed of according to national/international laws.

WARNINGS AND PRECAUTIONS

REAGENT 2 **WARNING:** ***H319*** *causes severe eye irritation.* ***H315*** Causes skin irritation.
REAGENT 1 and STANDARD: not classified as dangerous.

REFERENCES

1 Talke H., Schubert G.E. "Klin. Wochenschr.", 174 (1965)
2 Tiffany T.O. et al., "Clin. Chem.", 18, 829 (1972)
3 Kaplan LA, Pesce AJ: "Clinical Chemistry", Mosby Ed. 1989

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KEY SYMBOLS

IVD	In Vitro diagnostic medical device
LOT	batch number
REF	catalogue number
	temperature limits
	use by
	caution
	consult accompanying documents

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