



CREATININE

Kinetic colorimetric determination of Creatinine
in biological fluids



ORDER INFORMATION

REF	Kit size
GA4450 00	5x50 + 5x50 ml
KL4450 00	8x40 + 8x40 ml

INDICATION

Creatinine is a metabolic waste product formed due to non-enzymatic dehydration of Creatine derived from Creatine phosphoric acid. Determination of serum or urinary Creatinine is a useful diagnostic tool for kidney diseases such as acute chronic nephritis and other disorders such as urethropraxis, mercurialism and nephrosis.

METHOD PRINCIPLE

Serum and urine Creatinine reacts with Picric acid in alkaline solution yielding a yellow-orange coloured compound. The intensity of the colour is directly proportional to the Creatinine concentration present in the sample.

COMPOSITION

REAGENT A:

Sodium hydroxide 1.25 mmol/l
Corrosive R34; S(1/2-)26-37/39-45.

REAGENT B:

Picric acid 20.5 mmol/l

STANDARD:

1x5 ml Creatinine 2 mg/dl
Verified against NIST reference material.

PREPARATION OF REAGENTS

Bireagent procedure:

The reagents are liquids ready to use.

Monoreagent procedure:

Mix 1 part of Reagent A and 1 part of Reagent B to obtain the working reagent (ex. 10 ml of RA + 10 ml of RB).

Storage and stability

Store at room temperature (15-25 °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 7 days at 2-8 °C.

ANCILLARY EQUIPMENT

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

SAMPLES

Serum, urine 24h. Stable 24 hours at 2-8 °C.
Dilute urine 1:25 with distilled water.

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 Creatinine concentration. Check that the values obtained are within the reference range provided.

ANALYTICAL PROCEDURE

Allow the reagents to reach working temperature before using.

Bireagent procedure

Pipette into disposable or well clean cuvettes:

	Sample	Standard
Serum or diluted urine	100 µl	-
Standard	-	100 µl
Reagent A	500 µl	500 µl
Mix and incubate for 5 minutes, then add:		
Reagent B	500 µl	500 µl
Mix and, after 10 seconds , read absorbance (A₁) at 490-510 nm ; read again after 1 minute the absorbance (A₂). Analysis of samples and standards have to be executed at the same temperature.		

Monoreagent procedure

Pipette into disposable or well clean cuvettes:

	Sample	Standard
Serum or diluted urine	100 µl	-
Standard	-	100 µl
Working reagent	1000 µl	1000 µl
Mix and, after 10 seconds , read absorbance (A₁) at 490-510 nm ; read again after 1 minute the absorbance (A₂). Analysis of samples and standards have to be executed at the same temperature.		

CALCULATION OF RESULTS

Calculate ΔA ($A_2 - A_1$) for all the samples and for the standard.

Serum:

$$\text{mg/dl} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times 2$$

Urine:

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

Urine: (when 24 hours diuresis is known)

$$\text{g/24h} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times 0.5 \times \text{l/24h}$$

$$\text{mg/kg/24h} = \frac{\text{urine creatinine, g/24h}}{\text{body mass (Kg)}} \times 1000$$

Clearance: (when 24 hours diuresis is known)

$$\text{ml/min.} = \frac{\text{urine creatinine, mg/dl} \times \text{ml/24h}}{\text{serum creatinine, mg/dl} \times 1440}$$

Note:

1. For creatinine values higher than 6 mg/dl, repeat the determination using sample diluted 1:5 in saline solution; multiply the result by 5.
2. For the children the body surface (m^2) must be taken into account in the calculation, multiplying the previous result by $1.73/\text{m}^2$.
3. This method can be applied, by proportionally varying the working volumes, to all automatic instruments where the serum works as starter.

REFERENCE VALUES

Sample	Subjects	Range	Units
Serum	Male	0.6 ÷ 1.3	mg/dl
	Female	0.5 ÷ 1.2	mg/dl
Urine	Adults	1.3 ÷ 1.8	g/24h
	Male	20 ÷ 26	mg/Kg/24h
	Female	14 ÷ 24	mg/Kg/24h
Clearance	Male	107 ÷ 139	ml/minute
	Female	87 ÷ 107	ml/minute

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 three samples at different Creatinine concentrations. The obtained results are reported in the following table:

Sample	Mean (mg/dl)	Within Run		Between Run	
		SD	%CV	SD	%CV
Serum 1	1.72	0.02	1.2	0.13	7.6
Serum 2	3.05	0.03	1.0	0.26	8.5
Serum 3	4.52	0.04	0.9	0.37	8.2

Linearity

The assay is linear up to 6 mg/dl.

Sensitivity

Test sensitivity, in terms of limit of detection, is 0.1 mg/dl.

Correlation

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

$$y = 1.0534x + 0.6955 \text{ mg/dl} \quad r = 0.9541$$

Interferences

Hemoglobin > 200 mg/dl
 Bilirubin > 20 mg/dl
 Triglycerides > 1000 mg/dl

PRECAUTIONS IN USE

Reagent A is harmful (Corrosive).

Refer to Safety Data Sheet.

Reagent B and Standard are not considered harmful according to 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.

BIBLIOGRAPHY

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4. NCCLS Document, "Procedures for the collection of arterial blood specimens", Appr. Std., 3rd Ed. (1999).
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