



CREATININE Enzymatic

End point colorimetric determination of Creatinine
in serum, plasma and urine



ORDER INFORMATION

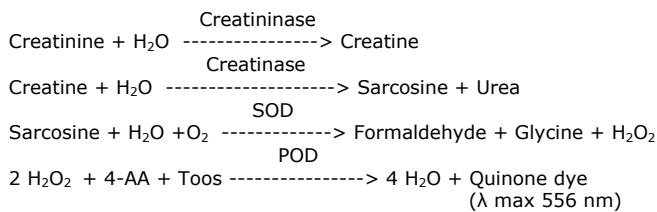
REF	Kit size
GA4420 00	1x45 + 1x15 ml
KL4420 00	1x45 + 1x15 ml
BK4420 00	2x(45+15 ml)

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

The enzymatic assay for creatinine involves a series of coupled enzymatic reactions including creatininase enzymatic conversion of creatinine into the product creatine which is converted to sarcosine by creatine amidinohydrolase (creatinase), followed by oxidation of sarcosine by sarcosine oxidase (SOD) producing hydrogen peroxide. In the presence of peroxidase (POD) the hydrogen peroxide is quantified at 550 nm by the formation of a colored dye.



Any endogenous creatine present in the sample is removed by creatinase and sarcosine oxidase during preincubation.

COMPOSITION

REAGENT A:

Creatinase	12-60 IU/ml
Sarcosine oxidase	4-17 IU/ml
TOOS	0.07-0.21 mg/ml

REAGENT B:

Creatininase	135-670 IU/ml
Peroxidase	20 kU/l
4-AA	0.3-0.9 mg/ml

STANDARD:

Creatinine	1x2 ml 2 mg/dl (177 µmol/l)
------------	--------------------------------

Preparation

The reagents are liquids ready to 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.

ANCILLARY EQUIPMENT

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

SAMPLES

Fresh serum, plasma (heparin, EDTA), urine 24h.
Dilute urine 1:10 with saline solution.

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.

Pipette into disposable or well clean cuvettes:

	Blank	Standard	Sample
Reagent A	270 µl	270 µl	270 µl
Distilled H ₂ O	8 µl	-	-
Standard	-	8 µl	-
Sample	-	-	8 µl

Mix and incubate for **5 minutes** at **37 °C**.
Read the absorbance (**A₁**) of the standard and samples at **550 nm** against water. Then add:

	Blank	Standard	Sample
Reagent B	90 µl	90 µl	90 µl

Mix and incubate for **5 minutes** at **37 °C**.
Read the absorbance (**A₂**) of the standard and samples at **550 nm** against water.

CALCULATION OF RESULTS

Calculate $\Delta A (A_2 - A_1)$ for all the samples and for the standard.

Serum:

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

Urine:

$$\text{mg/dl} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times 20$$

Urine: (when 24 hours diuresis is known)

$$\text{g/24h} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times 0.2 \times \text{l/24h}$$

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

Clearance: (when 24 hours diuresis is known)

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

Conversion factor

$$\text{Creatinine [mg/ml]} \times 88.4 = \text{Creatinine [µmol/l]}$$

$$\text{Creatinine [µmol/l]} \times 0.0113 = \text{Creatinine [mg/ml]}$$

Note:

For creatinine values exceeding the linearity limit repeat the determination using sample diluted with saline solution; multiply the result by dilution factor used.

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

Serum

Precision has been evaluated by testing four serum specimens at different creatinine concentration with two runs per day with duplicate over 20 working days. The obtained results are reported in the following tables.

Serum: within-run precision

Sample	n	Mean (mg/dl)	SD	%CV
Serum # 1	80	0.74	0.015	2.1
Serum # 2	80	1.38	0.015	1.1
Serum # 3	80	4.04	0.029	0.7
Serum # 4	80	10.28	0.015	0.1

Serum: total precision

Sample	n	Mean (mg/dl)	SD	%CV
Serum # 1	80	0.74	0.022	3.0
Serum # 2	80	1.38	0.026	1.9
Serum # 3	80	4.04	0.058	1.4
Serum # 4	80	10.28	0.140	1.4

Urine

Within-run precision has been evaluated by testing 21 replicates of three commercial urine controls at different creatinine concentration in the same run.

For total precision, 2 runs of each commercial urine control were performed consecutively for 5 days. The samples were diluted ten-fold with 0.9% saline and tested for creatinine values. The values were multiplied by the dilution factor (i.e. 10) to obtain the final results indicated below.

Urine: within-run precision

Sample	n	Mean (mg/dl)	SD	%CV
Urine # 1	21	29.09	0.100	0.36
Urine # 2	21	87.10	0.270	0.31
Urine # 3	21	196.70	0.900	0.46

Urine: total precision

Sample	n	Mean (mg/dl)	SD	%CV
Urine # 1	20	29.86	0.790	2.64
Urine # 2	20	87.70	0.670	0.76
Urine # 3	20	195.00	1.190	0.60

Linearity

The linearity of the procedure is 0.14-13.56 mg/dl in serum and 0.14-141.25 mg/dl in urine.

Sensitivity

Test sensitivity, in terms of limit of detection, is 0.14 mg/dl.

Correlation

Accuracy has been evaluated by assaying serum samples (range: 0.2-13.51 mg/dl) and urine samples (range: 0.14-141 mg/dl) with the present method and a legally marked creatinine assay. The regression curves calculated are the following:

Serum: $y = 0.9467x + 0.0643$ mg/dl $r = 0.9981$

Urine: $y = 1.005x - 0.2979$ mg/dl $r = 0.9969$

Interferences

The following substances produced less than 10% deviation at the listed concentrations:

Triglycerides	1000 mg/dl
Hemoglobin	500 mg/dl
Bilirubin	40 mg/dl
Bilirubin conjugate	30 mg/dl
Ascorbic acid	10 mg/dl

Certain drugs can sometimes cause abnormally elevated creatinine values.

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.

BIBLIOGRAPHY

1. Cook JGH. Factors influencing the assay of creatinine. *Ann. Clin. Biochem.* 1975; 12:219-232.
2. Heinegård D, Tiderström G. Determination of serum creatinine by a direct colorimetric method. *Clin. Chim. Acta.* 1973; 43:305-310.
3. Jaffe M. Ueber den nachweis der picrinsäure in normalem harnerzeugt und über eine neue reaction des kreatinins. *Z. Physiol. Chem.* 1886; 10:391-400.
4. Owen JA, Iggo B, Scandrett FJ, Stewart CP. The determination of creatinine in plasma or serum, and in urine; a critical examination. *Biochem. J.* 1954; 58:426-437.
5. Tobias GJ, McLaughlin RF, Hopper J. Endogenous creatinine clearance: a valuable clinical test of glomerular filtration and a prognostic guide in chronic renal disease. *N. Engl. J. Med.* 1962; 266:317-323.
6. Centers for Disease Control. Recommendations for prevention of HIV transmission in health-care settings. *MMWR* 1987;36 (suppl no. 2S):001.
7. Piero Fossati, Lorenzo Prencipe, and Giovanni Bertl. Enzymic Creatinine Assay: A New Colorimetric Method Based on Hydrogen Peroxide Measurement *CLINICAL CHEMISTRY* 29/8, 1494-1496 (1983).
8. Tietz NW, ed. *Clinical Guide to Laboratory Test*, 3rd ed. Philadelphia, Pa: WB Saunders Company 1995:186-188.
9. NCCLS Document, "Procedures for the collection of arterial blood specimens", Appr. Std., 3rd Ed. (1999).
10. EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC.

 **Manufacturer:**

MINIAS GLOBE DIAGNOSTICS SRL

Via Galileo Galilei 38 - Seggiano di Pioltello (Milan) Italy
Tel: + 39 02 929189 1 - Fax: + 39 02 929189 39

Met. GA442000.0 ing
Edition: 2010/05/10