

UROCHEM CREATININE KIT

(Alkaline Picrate method)

For the determination of Creatinine in serum and urine.
(For In-vitro Diagnostic Use Only)

Summary

Creatinine is the catabolic product of creatinine phosphate which is used by the skeletal muscle. The daily production depends on muscular mass and it is excreted out of the body entirely by the kidneys. Elevated levels are found in renal dysfunction, reduced renal blood flow (shock, dehydration, congestive heart failure) diabetes acromegaly. Decreased levels are found in muscular dystrophy.

Principle

Picric acid in an alkaline medium reacts with creatinine to form an orange coloured complex with the alkaline picrate. Intensity of the colour formed is directly proportional to the amount of creatinine present in the sample.

Creatinine + Alkaline Picrate → Orange Coloured Complex

Reference values

	Serum	Urine, 24hrs. collection
Male	0.6 - 1.2 mg%	1.1 - 3.0 gm
Females	0.5 - 1.1 mg%	1.0 - 1.8 gm

It is recommended that each laboratory establish its own normal range representing its patient population.

Contents

	15 Tests	35 Tests
L1 : Picric Acid Reagent	60 ml	140 ml
L2 : Buffer Reagent	5 ml	12 ml
S : Creatinine Standard (2mg/dl)	5 ml	5 ml

Storage / stability

All reagents are stable at R.T. till the expiry mentioned on the labels

Reagent Preparation

Reagents are ready to use. Do not pipette with mouth.

Sample material

Serum or Urine.

Creatinine is stable in serum for 1 day at 8°C

Urine of 24 hours collection is preferred. Dilute the specimen 1:50 with distilled / deionised water before the assay.

Procedure

Wavelength / filter	: 520 nm (H) 546 nm / Green
Temperature	: R.T.
Light path	: 1 cm

Deproteinization of specimen :

Pipette into a clean dry test tube

Picric acid reagent (L1)	2.0 ml
Sample	0.2 ml

Mix well and centrifuge at 2500 - 3000 rpm for 10 min. to obtain a clear supernatant

Colour development :

Pipette into clean dry test tubes labelled as Blank (B), Standard (S), and Test (T) :

Addition Sequence	B (ml)	S (ml)	T (ml)
Supernatant	-	-	1.1
Picric Acid Reagent (L1)	1.0	1.0	-
Distilled water	0.1	-	-
Creatinine Standard (S)	-	0.1	-
Buffer Reagent (L2)	0.1	0.1	0.1

Mix well and keep the test tubes at R.T. for exactly 20 minutes. Measure the absorbance of the Standard (Abs.S), and Test Sample (Abs.T) against the Blank.

Calculations

$$\text{Creatinine in mg\%} = \frac{\text{Abs.T}}{\text{Abs.S}} \times 2.0$$

$$\text{Urine Creatinine in gm/Lit.} = \frac{\text{Abs.T}}{\text{Abs.S}} \times 1.0$$

$$\text{Urine Creatinine gm/24Hrs.} = \text{Urine Creatinine in gm/L} \times \text{Vol. of urine in 24 Hrs.}$$

Linearity

The procedure is linear upto 8 mg% of creatinine.

If values exceed this limit, dilute the sample with distilled water and repeat the assay. Calculate the value using the proper dilution factor.

Note

Maintain the reaction time of 20 min. as closely as possible since a longer incubation causes an increase in the values due to the reaction of pseudochromogens.

The determination is not specific and may be affected by the presence of large quantities of reducing substances in the sample.

The reaction is temperature sensitive and all the tubes should be maintained at a uniform temperature.

References

Bones, R.W. et al. (1945) J. Biol. Chem. 158, 581.

Toro, G. et al. (1975) Practical Clinical Chem. P. : 154.

System Parameters

Reaction	: End Point	Interval	: —
Wavelength	: 520 nm	Sample Vol.	: 0.20 ml
Zero Setting	: Reagent Blank	Reagent Vol.	: 1.10 ml
Incub. Temp.	: R.T.	Standard	: 2 mg/dl
Incub. Time	: 20 min.	Factor	: —
Delay Time	: —	React. Slope	: Increasing
Read Time	: —	Linearity	: 8 mg/dl
No. of read.	: —	Units	: mg/dl

0.30 ml
 Blank - 71417
 T - 04877
 S - 0.2877
 R

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