

Creatinine

Alkaline Picrate Method

Code : 10007 (3x100 ml)

(For the analyser/Colorimetric estimation of Creatinine in Serum & Urine)

In VITRO USE Only.

SUMMARY & EXPLANATION OF TEST :

Serum Creatinine determination is mainly used for the diagnosis of renal diseases. Creatinine is an endogenous NPN (NON-Protein Nitrogen) Waste product of the body excreted through kidneys. Creatinine, after filtration in the glomerulus, is not reabsorbed in the tubules and hence urine creatinine measures glomerular filtration rate (GFR). Urine creatinine determination is usually carried out as a part of creatinine clearance test.

Creatinine Kit is based upon the Jaffe's Alkaline picrate reaction. Bonses and Tausskay made a useful study of the best conditions for the development of color, which are incorporated in CREATININE Kit. Although the reaction is non-specific, the chromogen thus estimated is in parallel and near to true creatinine concentration. Slot observed that the color developed by creatinine faded rapidly after acidifying with sulfuric acid, whereas that of non-creatinine chromogens remained unaffected. Hence, while using CREATININE kit, in doubtful cases, the true creatinine concentration can be determined by measuring the absorbance difference before & after acidifying the chromogen with a drop of concentrated sulfuric acid. The absorbance difference measures the true creatinine concentration.

PRINCIPLE:

In alkaline medium picric acid reacts with Creatinine and produces a red colored complex, whose absorbance is proportional to creatinine concentration. Picric acid reagent has a dual role as a deproteinizing agent and as a reactant.

REAGENTS:

1. Picric Acid Reagent 2x100ml
2. Sodium Hydroxide 0.2N 100ml
3. Standard (2mg%) 10ml

The reagents are ready to use and usable upto the expiration date when stored at room temperature.

SAMPLE :

Serum, plasma or Urine can be used.

1. Urine should be of 24 hours collection. Dilute urine specimen 1:50 using distilled water before use.
2. Hemolysed or lipemic serum should not be used, as it may give erroneous results.
3. Creatinine in serum is stable for at least two days at room temperature and one week at 2-8°C or much longer at -20°C.

EXPECTED RANGE :

	Serum Creatinine	Urine Creatinine
Male	0.9-1.5mg%	1.1-3.0 gm/lit
Female	0.8-1.3mg%	1.0-1.8 gm/lit

LINEARITY:

This method is linear upto 20 mg%. Samples exceeding 20mg% should be diluted and reassayed. The result has to be multiplied by the dilution factor.

INSTRUCTIONS:

1. Picric acid reagent do not pipette by mouth.
2. Creatinine test kit is in vitro use only.
3. The filtrate obtained in the deproteinization of specimen should be clear.
4. Adhere to the reaction time of 20 minutes as closely as possible.

If the samples for analysis are allow to stand longer, there may be an increase in absorbance due to pseudochromogens.

DIRECTIONS FOR USE ON ANALYSERS :

Reaction Type	:	End Point with std.
Reaction Slope	:	Increasing
Wave Length	:	520nm (green filter)
Incubation Temp	:	Room Temperature
Incubation Time	:	20 min.
Standard	:	2mg%
Linearity	:	20mg%
Unit	:	mg%

PROCEDURE:

Step -I: Deproteinization of specimen :

Pipette into a clean dry test tube labelled (T)

	(T)
Serum/Diluted Urine	1.0 ml
Picric Acid Reagent (1)	6.0 ml

Mix Well and filter/centrifuge at 2000-3000 rpm for 10 minutes, to obtain a clear filtrate/supernatant.

Step - II: Color development:

Label three clean dry test tubes as Blank(B), Standard(S) and Test (T), Pipette in to each test tube as shown below :

	(B)	(S)	(T)
Filtrate/Supernatant from step-1	-	-	3.5ml
Standard (3)	-	0.5ml	-
Distilled Water	0.5ml	-	-
Picric Acid Reagent (1)	3.0ml	3.0ml	-
Sodium Hydroxide (2)	2.0ml	2.0ml	2.0ml

Mix well and allow to stand at R.T. for exactly 20 minutes and measure the absorbance of Blank(B), Standard(S), and Test(T) against distilled water on a photocolormeter with green filter or on a spectrophotometer at 520 nm.

CALCULATIONS:

- a) Serum creatinine in mg% : $\frac{A \text{ of (T) - A of (B)}}{A \text{ of (S) - A of (B)}} \times 2.0 \text{ (std. Conc)}$
- b) Urine creatinine in g/Lit : $\frac{A \text{ of (T) - A of (B)}}{A \text{ of (S) - A of (B)}} \times 1.0$
- c) Urine Creatinine in g/24 hrs. Urine collection : (b)x24 hrs.
Urine volume in litres.

NOTES:

- ★ Due to variations in inter - laboratory assay conditions, instruments and demography, it is recommended that each laboratory should establish its own normal range. To ensure adequate quality control, each run should include a normal and abnormal assayed controls. The assigned value of the control must be confirmed by this methodology.
- ★ Final diagnosis should be based on a co-relation of test results with other clinical observations / Diagnostic tools.

BIBLIOGRAPHY:

Bonses, R.W. & Tausskay, H.H. (1945) J. Biol. Chem. 158, 581. Slot, C. (1965), Scand J. Clin. Lab. Invest. 17, 381. Toro, G. & Ackermann, P. (1975), Practical Clinical Chemistry, P. 154.

Manufactured in India by :

M/s Excel Diagnostics Pvt. Ltd.

Plot NO. 89, Road No.8, ALEAP I.E., Near Pragathi Nagar, Opp. Kukatpally JNTU, Hyderabad - 500 090 (A.P.) INDIA.
E-mail : edpl@rediffmail.com Visit us at - www.exceldiag.com