CREATININE

ENZOPAK

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(ALKALINE PICRATE METHOD)

Reagent kit for quantitative estimation of CREATININE in serum or urine.

BACKGROUND & SYNOPSIS :

Creatinine is an end product of creatine metabolism which is a part of energy utilization mechanism in muscles. The quantity of creatinine generated depends marginally on the dietary protein intake. The serum creatinine level depends directly on the body muscle mass and is affected by intense muscular stress.

In 1886, Jaffe developed a method for the assay of creatinine based upon the reaction between creatinine and sodium picrate. In 1904, Folin employed this reaction for the quantitative estimation of creatinine, in urine. Since then, a number of modifications have been formulated to reduce interference from proteins and non-creatinine substances like keto-acids, glucose, ascorbic acid etc.

ENZOPAK Creatinine is a modified formulation of Jaffe's reaction which does not require deproteinization. The picrate and alkali reagents are formulated in such a way that while measuring the rate of reaction, interference from non-creatinine substances present in the sample is almost eliminated.

PRINCIPLE :

Creatinine present in the serum or urine reacts with alkaline picrate to form a colored complex. The rate of formation of colored complex is directly proportional to creatinine concentration. This rate of reaction (intensity of color produced) is measured photometrically at 510 nm and is compared with that of the standard.

Creatinine + Alkaline Picrate $\longrightarrow$ Creatinine Picrate Complex

DIAGNOSTIC SIGNIFICANCE :

Creatinine is a waste product formed in muscle from the high energy storage compound, creatine phosphate. The amount of creatinine produced is fairly constant and is primarily a function of muscle mass. Creatinine is removed from plasma by glomerular filtration and then excreted in urine without any appreciable resorption by the tubules.

Creatinine is used to assess renal function, however, serum creatinine levels do not start to rise until renal function has decreased by at least 50%. Congestive heart failure, shocks and mechanical obstruction of urinary tract may also contribute to an elevated level of serum creatinine. An elevated serum creatinine level due to obstruction may rapidly fall when the obstruction is removed by surgery.

Many times serum urea/creatinine ratio is used for assessment of kidney function and differential diagnosis. Creatinine clearance test is carried out for assessment of kidney function only.

REAGENT COMPOSITION:

Active Ingredients	Concentration
Reagent-1	
* Picric Acid	20 mmol/L
Reagent-2	
* Sodium Hydroxide	100 mmol/L
Reagent-3 Standard (2 mg/dl)	
Also contains non-reactive fillers and stabilizers.	

PRESENTATION:

	No. of Bottles
All the reagents to be stored at 2-8^{0}C	100 ml
• 1 CREATININE (PICRATE REAGENT)	1
• 2 CREATININE (ALKALI REAGENT)	1
• CREATININE STANDARD (2 mg/dl)	1

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PRECAUTION :

ENZOPAK Creatinine is for *IN-VITRO* diagnostic use only. Avoid contact of the reagents with skin, eyes and clothes. Use of automated pipetting devices.

Reagent contains Sodium azide. DO NOT INGEST.

WORKING REAGENT PREPARATION:

Prepare working reagent by combining one volume of 1 CREATININE with one volume of 2 CREATININE. Mix well before use.

REAGENT STORAGE AND STABILITY:

All the reagents included in the kit are stable at 2-8^{0}C until the expiry date printed on the label.

The working reagent is stable at 2-8^{0}C for three months.

SPECIMEN COLLECTION:

Fresh, clear serum with no hemolysis is the specimen of choice. Plasma prepared using heparin as an anticoagulant may also be used.

REACTION PARAMETERS :

- Type of Reaction : Fixed Time/Two Point / Initial Rate
- Wavelength : 510 nm.
- Flowcell/Cuvette Temperature : 30^{0}C
- Delay Time : 20 seconds
- Interval : 60 seconds
- Measuring Time : 80 seconds
- No. of readings : 2
- Standard/Sample Volume : 100 microliters (0.1 ml).
- Standard Concentration : 2 mg/dL
- Working Reagent Volume : 1.0 ml.
- Light Path : 1.0 cm.
- Zero setting with : Distilled Water.

Note : For instruments using cuvette capacity of 2.5 ml, use sample and standard volume 200 microliters (0.2 ml) and working reagent 2.5 ml.

PROCEDURE :

PIPETTE INTO TEST TUBES	STANDARD (ST)	TEST (TS)
• WORKING REAGENT (ml)	1.0	1.0
• STANDARD (ml)	0.1	-
• SAMPLE (ml)	-	0.1

Mix and aspirate. Record the absorbance of Standard (ST) and Test (TS) at 20 seconds (ST_1, TS_1) and again at 80 seconds (ST_2, TS_2) at 510 nm, against distilled water.

TEST RESULTS:

$$\text{Creatinine concentration} = \frac{(TS_2 - TS_1)}{(ST_2 - ST_1)} \times 2$$

To convert (mg/dl) to micromol/liter, use the following equation; micromol/liter = (mg/dl) x 88.5

INTERFERENCE :

As per studies carried out for interference, following results were obtained.

- No Interference from Hemoglobin upto 50 mg/dl.
- No Interference from free Bilirubin upto 12 mg/dl.
- No Interference from Lipemic (Measured as Triglycerides) upto 500 mg/dl.

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NORMAL VALUES : (FOR SERUM)

Serum Creatinine : MEN : 0.9 - 1.4 mg/dl
WOMEN : 0.6 - 1.2 mg/dl

LINEARITY :
This method is linear upto 12 mg/dl. For sample values higher than linearity limit, dilute the samples suitably with 0.9 % saline and repeat the assay. Apply dilution factor to obtain test results.

CREATININE ESTIMATION IN URINE :
For Creatinine estimation in urine, dilute the sample suitably with distilled water and follow the procedure to calculate test results by applying dilution factor. Dilution of 1:15 to 1:100 is suggested.

Creatinine concentration in urine (gms/lit)

$$= \frac{(TS_2\text{-}TS_1)}{(ST_2\text{-}ST_1)} \times 2 \times \frac{\text{dilution factor}}{100}$$

NORMAL VALUES: (FOR URINE)
Urine Creatinine : MEN : 1.0 – 2.0 gms/24 hrs.
: WOMEN : 0.8 – 1.5 gms/24 hrs.

REFERENCE :
KAPLAN A., SZABO, L.L., Clinical Chemistry:
Interpretation and Techniques, Lea and Febiger, Philadelphia (1983).

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