CREATININE (End Point)

(Jaffe Method)

CHEMPAK

Last update 09-2020

Ref. CC3-CRE.08M, 120 ml

INTENDED USE
Reagent kit for quantitative estimation of Creatinine in serum.

INTRODUCTION
Creatinine is an end product of creatine metabolism which is 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 activity.

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 determination of creatinine in urine. Slot described a procedure for creatinine determination using absorbance reading taken before and after the addition of an acid reagent. This method of Creatinine for serum and urine is formulated on this principle and is relatively free from interference due to non-creatinine substances.

DIAGNOSTIC SIGNIFICANCE
Creatinine is a waste product formed in muscles 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 don't start to rise, until renal function has decreased by at least 50%. Congestive heart failure, shock and mechanical obstruction of urinary tract may also contribute to an elevated level of serum creatinine. Surgical removal of obstruction may result in a rapid fall of serum creatinine to normal levels.

PRINCIPLE
Acid reagent differentiates colour developed using Jaffe's reagents by (1) Creatinine + reactive non-creatinine substances and (2) reactive non-creatinine substances.

Colours developed before and after addition of acid reagent is measured photometrically at 510 nm (500 to 530 nm or green filter) and the difference is proportional to creatinine concentration.

Creatinine + Alk. Picrate ⟶ Creatinine Picrate Complex + Non-creatinine Complexes

Creatinine Picrate Complex + Non creatinine Complexes } Acid Reagent ⟶ { Non-Creatinine Complex + De-colorized creatinine & Picrate

PRESENTATION

	No. of Bottles 120 ml
• 1 Creatinine (Picrate Reagent)	1
• 2-Creatinine (Alkali Reagent)	1
• 3-Creatinine (Acid Reagent) Store at Room Temperature	1
• Creatinine Standard, (2 mg/dl.) Store at 2 – 8°C (Provided Separately)	1

FINAL REAGENT COMPOSITION

Active Ingredients	Concentration
• Picric Acid	20 mmol/L
• Sodium Hydroxide	100 mmol/L
• Glacial Acetic Acid	2500 mmol/L

Creatinine Standard (2 mg/dl)
Also contains non-reactive fillers and Stabilizers.

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

PREPARATION OF WORKING REAGENT
Prepare working reagent by mixing equal volumes of reagents 1 Creatinine and 2 Creatinine as per daily requirements.

REAGENT STORAGE AND STABILITY
All the reagents included in the kit are stable at room temperature until the expiry date printed on the label.

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	:	Two Step End Point
• Wavelength	:	510 nm
• Flowcell Temperature	:	30^0 C / 37^0 C
• Std. Concentration	:	2 mg/dl
• Sample Volume	:	200 µl (0.20 ml)
• Reagent Volume	:	2.5 ml
• First Incubation Time	:	15 min. at 37^0 C
• Reagent 3 Volume	:	100 µl (0.10 ml)
• Second Incubation	:	5 min. at 37^0 C
• Light Path	:	1.0 cm
• Zero setting with	:	Distilled Water

TEST PROCEDURE
DEPROTEINIZATION OF SAMPLE IS NOT REQUIRED

Pipette Into Test Tubes	STANDARD	TEST
Working Reagent (ml)	2.5	2.5
Sample (ml)	-	0.2
Standard (ml)	0.2	-

Mix well and incubate for 15 minutes at 37^0C (or 25 to 30 minutes at room temperature).

Read absorbance of standard (STD_1) and test (TS_1) at 510 nm (505-530 nm or with GREEN filter) against distilled water.

3 Creatinine (ml)	0.1	0.1

Mix well and keep for 5 minutes at 37^0C (or ten minutes at room temperature) and read absorbance of standard (STD_2) and test (TS_2) at 510 nm (505-530 nm or with GREEN filter) against distilled water.

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TEST RESULTS

$$\text{Creatinine concentration (mg/dl)} = \frac{TS}{STD} \times 2$$

Where
TS = Corrected absorbance of sample = $TS_1 - TS_2$
STD = Corrected absorbance of standard = $STD_1 - STD_2$

NORMAL VALUES
(For Serum)
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 sample suitably with 0.9 % saline and repeat the assay. Apply proper dilution factor to calculate the final 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. A dilution of 1:15 or 1:100 is suggested

$$\text{Creatinine concentration in urine (gms/lit)} = \frac{TS}{STD} \times 2 \times \frac{\text{Dilution factor}}{100}$$

NORMAL VALUES
(For Urine)
Men : 1.0 – 2.0gms/24 hours
Women : 0.8 – 1.5gms/24 hours

AUTOMATED APPLICATION
For automated instruments, use of ENZOPAK Creatinine is recommended.

REFERENCES

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

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