CONCEPT DIAGNOSTICS
ONTARIO, CALIFORNIA 91761 USA

CRESTLINE SCIENTIFIC CORPORATION
2902 Finlandia St., cor Copernico St.,
San Isidro Village, Makati City
Tel: 8875777 Fax: 8875778

CREATININE REAGENT SET (KINETIC PROCEDURE)

CREATININE REAGENT SET
Kinetic method for the quantitative determination of creatinine in serum

INTRODUCTION
Creatinine, an anhydride of creatine, is a waste product formed by the spontaneous dehydration of kidneys.[1] Most of the creatinine is found in muscle tissue where it is present as creatine phosphate and serve, as a high energy storage reservoir for conversion to ATP. Independent of diet serum creatinine concentrations depends almost entirely upon its excretion rate by the kidneys. For this reason its elevation is highly specific for kidney diseases.[2] The assay of creatinine has been based on the reaction of creatinine with alkaline picrate as described by Jaffe. Further modifications have developed the Jaffe reaction into a kinetic assay that is fast, simple and avoid interference.

PRINCIPLE

Creatinine + Sodium Picrate $\xrightarrow{\text{Alkali}}$ Creatinine - Picrat complex (yellow-orange)

Creatinine reacts with picric acid in alkaline conditions to form a color complex which absorbs at 510 nm. The rate of formation of color is proportional to the creatinine concentration in the sample.

REAGENTS
1. Creatinine Picric Acid Reagent: a solution containing 10 mM picric acid.
2. Creatinine Sodium Hydroxide: a solution containing 240 mM. sodium hydroxide.
3. Creatinine standard (5 mg/dl): A solution containing creatinine in hydrochloric acid with preservative.

PRECAUTIONS
1. This reagent is for "in vitro" diagnostic use only.
2. Creatinine Picric Acid Reagent is a strong oxidizing agent. Avoid contact with skin. **WIPE ANY SPILLAGE, SINCE PICRIC ACID IS EXPLOSIVE.**
3. Creatinine Sodium Hydroxide is an alkali.

REAGENT PREPARATION
Combine equal volumes of Creatinine Picric Acid Reagent and Creatinine Sodium Hydroxide, mix well.

REAGENT STORAGE
1. Both reagents are stored at room temperature (18 - 25°C).
2. Combined (working) reagent is stable for up to one (1) month.

REAGENT DETERIORATION
The reagent should be discarded if:
1. Turbidity has occurred; turbidity may be a sign of contamination.
2. The reagent fails to meet linearity claims or fails to recover control value, in the stated range.

SPECIMEN COLLECTION AND STORAGE
1. Serum is recommended.
2. Creatinine in serum is stable for twenty-four (24) hours at refrigerated temperatures (2 - 8°C) and for several months when frozen (-20°C) and protected from evaporation and contamination.
3. 24 hour urine specimens must be preserved with 15 grams of boric acid.

INTERFERENCES
A number of substances affect the accuracy of creatinine determination. See Young, et al[3] for a comprehensive list.

MATERIALS PROVIDED
1. Creatinine Picric Acid Reagent.
2. Creatinine Sodium Hydroxide.
3. Creatinine Standard.

MATERIALS REQUIRED BUT NOT INCLUDED
1. Pipetting devices.
2. Timer.
3. Heating bath/rack.
4. Test tube/rack.
5. Vessel for combining reagents (glass or plastic).
6. Spectrophotometer with a temperature controlled cuvette.

PROCEDURE (AUTOMATED)
Refer to specific instrument application instructions.

PROCEDURE
1. Combine equal volume, of Creatinine Picric Acid Reagent and Creatinine Sodium Hydroxide. Mix well.
2. Set the spectrophotometer cuvette temperature to 37°C.
3. Pipette 1.0 ml of working reagent into test tubes.
4. Zero spectrophotometer with the reagent blank at 510 nm. (Wavelenghts range: 500 - 520)
5. Add 0.05 ml (50 µl) of sample to reagent, mix and immediately place into cuvette.
6. After exactly thirty (30) seconds read and record the absorbance.(A_1).
7. At exactly sixty (60) seconds after the A_1 reading, read and record the absorbance (A_2) (i.e. time elapsed between A_1 - A_2 is sixty (60) seconds.)
8. Calculate the change in absorbance (ΔAbs./min.) by subtracting (A_2 - A_1). See "Calculations."

* USE MULTI PURPOSE CALIBRATOR TO REPLACE STANDARD.

ALTERNATE VOLUMES
If the spectrophotometer in use require a volume greater than 1.0 ml for accurate reading, use 0.2 ml (200 ul) sample to 3.0 ml reagent. Perform as above.

CALCULATIONS

The creatinine value of the unknown is determined by comparing its absorbance change with that of a known standard.

$$\text{mg/dl} = \frac{\Delta \text{ Abs.(unknown)}}{\Delta \text{ Abs.(standard)}} \times \text{Concentration of Standard}$$

Where:

ΔAbs. = Absorbance change between readings (A_2-A_1)

SAMPLE CALCULATION

If:	ΔAbs./Unknown	= 0.020
	ΔAbs./Standard	= 0.050
	Conc. of Standard	= 5 mg/dl

Then

$$\frac{0.020}{0.050} \times 5 = 2.0 \text{ mg/dl creatinine}$$

PROCEDURE LIMITATIONS

Albumin at a concentration of 10.0 mg/dl contributes 0.2 mg/dl to the creatinine value, moderate hemolysis (0.2 gm/dl Hgb), grossly icteric and lipemic samples will give elevated results. Acetoacetate above 10 mg/dl will interfere with the results.

CALIBRATION

Use the aqueous standard provided.

QUALITY CONTROL

The integrity of the reaction should be monitored by use of normal and abnormal control sera with known creatinine values.

EXPECTED VALUES[4]

Serum:	Male	0.9 - 1.5 mg/dl
	Female	0.7 - 1.37 mg/dl

PERFORMANCE CHARACTERISTICS

Linearity: 25 mg/dl

Comparison: A study performed between this procedure and a similar kinetic procedure yielded a correlation coefficient of 0.99 with a regression equation of y = 0.96x + 0.06. Serum and control samples used in the study had creatinine values ranging from 0.9 to 8.3 mg/dl.

Precision:

A. Within Run

Mean	S.D.	C.V.%
1.9	0.05	2.6
8.2	0.6	7.3

B. Run to Run

Mean	S.D.	C.V.%
2.0	0.2	10.0
8.2	0.4	4.6

REFERENCES

1. Henry, J.B., *Clinical Diagnosis and Management by Laboratory Method*, 16th ed. Saunders, Philadelphia, PA, p. 263, (1974).
2. Vasilades, *J. Can. Chem.* 22:1664 (1976).
3. Young, D.S., et *al., Can. Chem.* 21 (1975).
4. Tietz, N.W., *Fundamentals of Clinical Chemistry, W.* Saunders, S, Phila, p.1211 (1976).

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