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CREATININE REAGENT SET

(DIRECT ENDPOINT PROCEDURE)
Catalog Number: BQ035CR

Creatinine reagent is used for the quantitative determination of creatinine in human 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 serves 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 avoids interferences. In the endpoint method, acetic acid is used to destroy the creatinine picrate complex, resulting in a loss of color, the non creatinine serum constituents retain their picrate derived colors and thus, the differences in absorbencies gives the creatinine concentration.[3,4] This procedure is further modified by Heinegard and Tiderstrom[3] to eliminate the interference without acid treatment.

PRINCIPLE

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

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. In the endpoint method the difference in absorbance measurements after color formation yields a creatinine value corrected for interfering substances.

REAGENTS

1. Creatinine Picric Acid Reagent: a solution containing 10mM picric acid.
2. Creatinine Buffer Reagent: a solution containing 10 mM sodium borate, 240 mM sodium hydroxide, and surfactant.
   ***Important Note:** If the Creatinine Buffer Reagent has been subjected to cold temperatures white precipitate may form. Warm reagent to 37 °C with agitation to dissolve all the precipitate before use.
3. Creatinine standard (5.0 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 buffer reagent is an alkali. Avoid ingestion and contact.

REAGENT PREPARATION

Combine equal volumes of Creatinine Picric Acid Reagent and Creatinine Buffer Reagent, 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) week.

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 values 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 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[5] for a comprehensive list.

MATERIALS PROVIDED

1. Creatinine Picric Acid Reagent.
2. Creatinine Buffer Reagent.
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)

Not available.

ASSAY PROCEDURE

1. Combine equal volumes of Creatinine Picric Acid Reagent and Creatinine Buffer Reagent, mix well.
2. Label test vial, reagent blank, standard, control, and unknown test tubes.
3. Pipette 3.0 ml of working reagent into test tubes.
4. Transfer 0.1 ml (100 µl) of sample to its respective tube, distilled water to reagent blank and mix.
5. Place all tubes in 37 °C heating bath for fifteen (15) minutes.
6. Set wavelength of the spectrophotometer at 510 nm and zero the instrument with the reagent blank. Read and record the absorbance of all tubes. (Wavelength range: 500-520nm).
7. Calculate creatinine value. See "calculations".

* MULTI PURPOSE CALIBRATOR MAY BE USED TO REPLACE STANDARD.

ALTERNATE VOLUMES

If the spectrophotometer in use requires a volume less than 3.0 ml for accurate reading, use 0.05 ml (50 µl) sample to 1.0 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.

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

Where:
Abs. = Absorbance

EXAMPLE:
If: Abs./Unknown = 0.040
Abs./Standard = 0.150
Conc. of Standard = 5.0 mg/dl
Then:

$$\frac{0.040}{0.150} \times 5.0 = 1.3 \text{ mg/dl creatinine}$$

PROCEDURE LIMITATIONS
Albumin at a concentration of 10.0 gm/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.(Also can use multipurpose calibrator to replace standard)

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

EXPECTED VALUES[6]

Serum:	Male	0.9 - 1.50 mg/dl
	Female	0.7 - 1.37 mg/dl

PERFORMANCE CHARACTERISTICS
1. Linearity: 25 mg/dl
2. 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.
3. Precision:

Within Run

Mean	S.D.	C.V. %
1.9	0.05	2.6
8.2	0.60	7.3

Run-to-Run

Mean	S.D.	C.V. %
2.0	0.20	10.6
8.0	0.40	4.6

REFERENCES

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5. Young. D.S.. et *al., Clin. Chem.* 21(1975).
6. Tietz. N.W.. *Fundamentals of Clinical Chemistry*. W.B. Saunders. R.S., Phila. p. 1211(1976).