TECO DIAGNOSTICS
1268 N. Lakeview Ave.
Anaheim, CA 92807
1-800-222-9880

CREATININE REAGENT SET (KINETIC OR MANUAL ENDPOINT PROCEDURE)

INTENDED USE
Kinetic and endpoint methods 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 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 absorbances gives the creatinine concentration.[3,4]

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 and after acidification yields a creatinine value corrected for interfering substances.

REAGENTS
1. Creatinine Picric Acid Reagent: a solution containing 10 mM 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, a white precipitate may form. Warm reagent to 37°C with agitation to dissolve all the precipitate before use.
3. Creatinine standard (5 mg/dl): A solution containing creatinine in hydrochloric acid with preservative.
4. Acetic acid reagent: glacial acetic acid.

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. Acetic acid is acid. 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.
4. Acetic Acid Reagent.

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

PROCEDURE (AUTOMATED)
Refer to specific instrument application instructions.

KINETIC PROCEDURE
1. Label test tubes: Blank, Standard, Control, Patient, etc…
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. (Wavelength range: 500-520 nm).
5. Add .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).
8. Calculate the change in absorbance (ΔAbs/min.) by subtracting ($A_2 - A_1$). See "Calculations".

* TC - MULTI PURPOSE CALIBRATOR MAY BE USED TO REPLACE STANDARD.

ALTERNATE VOLUMES
If the spectrophotometer in use requires a volume greater than 1.0 ml for accurate reading, use 0.2 ml (200 µl) 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.

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

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

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

KINETIC 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.

MANUAL ENDPOINT 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 ± 5 nm and zero the instrument with the reagent blank. Read and record the absorbance of all tubes. These absorbances are the A_1 readings.
7. Add 0.1ml (l00 µl) of acetic acid reagent to each tube, mix and let stand for five (5) minutes at room temperature.
8. Re-zero the instrument with reagent blank. Read and record absorbances of all tubes. These absorbances are the A_2 readings.

* TC – MULTI PURPOSE CALIBRATOR MAY BE USED TO REPLACE STANDARD.

CALCULATION OF RESULTS
Use the absorbance readings of the standard and unknown to calculate creatinine values as follows:

$$\frac{\text{(A1 - A2) Unknown}}{\text{(A1 - A2) Standard}} \times \text{conc. of standard} = \text{creatinine (mg/dl) in unknown}$$

EXAMPLE OF CALCULATION
Assume the value of the standard to be 5.0 mg/dl and that it gave an A_1 of 0.23 and A_2 of 0.0 respectively, while unknown gave an A_1 of 0.28 and A_2 of 0.18. The creatinine concentration of the unknown may then be calculated as follows:

$$\frac{0.28 - 0.18}{0.23 - 0.00} \times 5.0 \text{ mg/dl} = \frac{0.10}{0.23} \times 5.0 \text{ mg/dl} = 2.2 \text{ mg/dl}$$

MANUAL ENDPOINT PROCEDURE NOTE
1. The A_2 absorbance reading of the standard should be 0.0 because the addition of acid lowers the pH and destroys all of the creatinine picric complex.
2. The final color development is stable for 30 minutes at controlled room temperature (20 - 25°C).

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[6]

Serum:	Male	0.9 - 1.5 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:
   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

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Manufactured by:

TECO DIAGNOSTICS
1268 N. LAKEVIEW AVE.
ANAHEIM, CA 92807
U.S.A.

EMERGO EUROPE
Prinsessegracht 20
2514 AP The Hague
The Netherlands