

CREATININE
(For Serum or Urine)
Updated September 2013

I. References:

Creatinine-S™ Creatinine
Diagnostic Chemicals Ltd. – # 221-30

II. Principle:

According to the Diagnostic Chemicals Ltd. technique, creatinine is synthesized in the kidney, liver, and pancreas. It is transported in the blood to the muscle and brain where it is phosphorylated to phosphocreatine. Some free creatine in the muscle is converted to creatinine daily and the amount produced is proportional to muscle mass. The excretion rate is relatively constant in the absence of renal disease. This procedure is a modification of the Jaffe reaction. The rate of color change is proportional to the creatinine concentration at 500 nm.

III. Personal Protective Equipment:

1. Lab coat
2. Safety glasses/goggles
3. Latex gloves
4. Prepare reagents in designated fume hood

IV. Reagents:

1. Creatinine ($C_4H_7N_3O$) Base Reagent (R1)
 - a. Sodium hydroxide (NaOH), 0.25 mol/L
2. Creatinine Picrate Reagent (R2)
 - a. Picric acid ($C_6H_3N_3O_7$), 20.5 mM/L and surfactant
WARNING: Explosive when dry!
 - b. Store Creatinine Reagents 1 and 2 at room temperature (18-26°C). Reagents are stable until the expiration date shown on the labels.
WARNING: Reagent RA and R2 are corrosive and may cause burns. Reagent R2 is harmful by inhalation. Dried picric acid may be explosive if solution has dried out. Do not handle.
3. Creatinine Assay Reagent
 - a. Mix 4 volumes of Creatinine Base Reagent (R1) with 1 volume of Creatinine Picrate Reagent (R2)
 - b. Solution is stable for at least 2 weeks when stored at room temperature (18-26°C)
4. Creatinine Standard
 - a. 4 mg/dL or 354 μ mol/L Creatinine
5. Quality Control - The reliability of test results should be monitored with use of a control material with known creatinine levels in both the normal and abnormal ranges for each run of the assay.
 - a. Laboratory Urine Control
 - 1) Range may vary with levels purchased, check current control.

IV. Sample Preparation:

1. Serum or Plasma

- a. Serum or plasma should be separated as soon as possible from the red blood cells and stored in the refrigerator or freezer.

- b. Creatinine in serum or plasma is stable for at least 7 days at 2-8°C and indefinitely when stored frozen.
 - c. Serum samples require no dilution unless the creatinine concentration exceeds 25 mg/dL.
 - d. Plasma should be separated using heparin (0.2 mg/mL) or EDTA.
 - e. Hemolysis does not render serum unsuitable for the assay.
2. Urine
- a. Creatinine in urine is stable for 2 to 3 days at room temperature (18-26°C) and for at least 5 days at 2-8°C.
 - b. Urine should be diluted 1 part to 9 parts dH₂O, multiply the result by 10 to compensate for the dilution.

V. Standards/Calibration:

1. Each manual run of the procedure should be calibrated with a creatinine standard solution.
2. The calibrator's absorbance is used when calculating the results.

VI. Procedure:

1. Pipette 10 µL of either test, standard, control, or blank (dH₂O) in triplicate into wells of a microtiter plate
2. Add 200 µL of pre-warmed (37°C) Creatinine Assay Reagent to each well. Start timer when reagent is added to the last wells
3. Read (37°C) and record absorbance at 510nm after 30 and 90 seconds

VII. Calculations:

$$1. \text{Creatinine (mg/dL)} = \frac{\text{Test, 30 sec} - \text{Test, 90 sec}}{\text{Standard, 30 sec} - \text{Standard, 90 sec}}$$

*Concentration (mg/dL) of Creatinine Standard

NOTE: Under these conditions, the reaction is linear up to 180 seconds. Readings may be taken conveniently at other time intervals as long as time between readings is 60 seconds.

A sample with a change in absorbance of 0.193 would indicate a level of creatinine exceeding the linearity limit. Sample should then be diluted.

X 4*