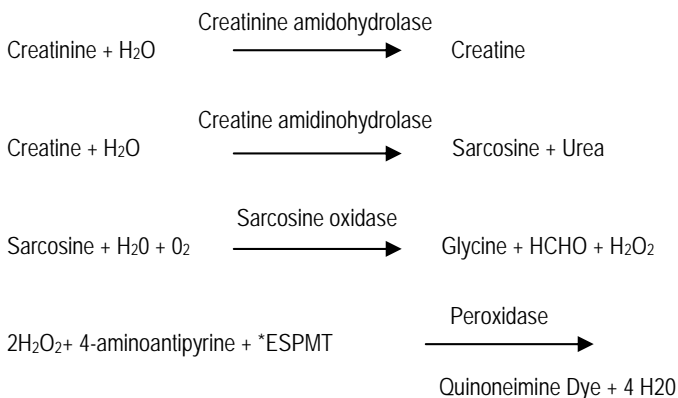# Creatinine (Enzymatic) Reagent Set

Intended Use
For the quantitative enzymatic determination of creatinine in serum and urine. For In Vitro Diagnostic Use Only.

Principle
Creatinine is a catabolic product of creatine, which is used in skeletal muscle contraction. The daily production of creatine, and subsequently creatinine, depends on muscle mass, which fluctuates very little. Creatinine is excreted entirely by the kidneys and therefore is directly proportional to renal excretory function. Thus with normal renal excretory function, the serum creatinine level should remain constant and normal. Only renal disorders, such as glomerulonephritis, pyelonephritis, acute tubular necrosis, and urinary obstruction, will cause an abnormal elevation in creatinine.[1]

The Pointe Scientific, Inc. method employs a two reagent system which eliminates interference by endogenous creatine and ascorbic acid.

$$\text{Creatinine} + H_2O \xrightarrow{\text{Creatinine amidohydrolase}} \text{Creatine}$$

$$\text{Creatine} + H_2O \xrightarrow{\text{Creatine amidinohydrolase}} \text{Sarcosine} + \text{Urea}$$

$$\text{Sarcosine} + H_2O + O_2 \xrightarrow{\text{Sarcosine oxidase}} \text{Glycine} + HCHO + H_2O_2$$

$$2H_2O_2 + \text{4-aminoantipyrine} + \text{*ESPMT} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine Dye} + 4\,H_2O$$

*ESPMT: N-ethyl-N-sulfopropryl-m-toluidine

Reagents
Creatinine Enzyme Buffer Reagent (R1): Good Buffer (pH 7.4) 25 mmol/L, Creatine amidinohydrolase > 25 KU/L, Sarcosine oxidase > 7 KU/L, Ascorbate oxidase > 4 KU/L, ESPMT 140 mg/L

Creatinine Enzyme Color Reagent (R2): Good Buffer (pH 7.3) 100 mmol/L, Creatinine amidohydrolase > 250 KU/L, Peroxidase > 5 KU/L, 4-aminoantipyrine 600 mg/L, ESPMT

Reagent Preparation
Reagents are provided as ready to use liquids.

Reagent Storage and Stability
Reagents are stable until expiration dates found on their labels when stored at 2-8°C.

Creatinine Standard is stable until the date of its expiration when properly stored at 15-30°C.

Specimen Collection and Storage
1. Serum: Remove specimen from clot promptly to prevent hemolysis.
2. Do not use fluoride or ammonium heparinate to collect sample.[2]

Sample Stability: Creatinine values have a reported stability of one day at 2-8°C, and several months when frozen (-20°C) and protected from evaporation and contamination. Store urine at 2 -8°C.[2]

Interferences
No interference was observed by ascorbic acid up to 200 mg/dL, hemoglobin up to 500 mg/dL, bilirubin-conjugate up to 32 mg/dL, and bilirubin-free up to 40 mg/dL. An extensive list of drugs or other agents interfering with creatinine methodologies has been reported by Young et al[3].

Materials Provided
1. Creatinine R1 Reagent
2. Creatinine R2 Reagent

Materials Required but not Provided
1. Spectrophotometer capable of absorbance readings at 550 nm
2. Constant temperature block or bath (37°C)
3. Temperature controlled cuvette well (37°C)
4. Interval timer
5. Accurate pipetting devices
6. Test Tubes
7. Vortex mixer
8. Creatinine Standard (C7513-STD) or Chemistry Calibrator (C7506-50)

Procedure (Manual)
1. Pipet into cuvettes labeled Bl (Blank), Std (Calibrator or Standard), and S (Specimen) the following volumes (µL).

	Bl	Std	S
Reagent 1 (R1)	270	270	270
Standard (Std)	–	6	–
Sample	–	–	6
Water	6	–	–

2. Mix, incubate for 5 min at 37°C, read absorbance A1.
3. Add 90µL of Reagent 2 (R2), mix and incubate for 5 min at 37°C, read absorbance A2.

Quality Control
Two (2) levels of control material with known Creatinine levels determined by this method, should be analyzed each day of testing.

Results
Values are derived by comparing the absorbance change of the specimen (S) with that of a standard (Std) and subtracting the (Bl) reading from both with samples and standard identically treated.

$$\text{Creatinine (mg/dL)} = \frac{[(AS_2 - AS_1) - (ABl_2 - ABl_1)] \times \text{Std Conc.}}{(AStd_2 - AStd_1) - (ABl_2 - ABl_1)}$$

Creatinine (Enzymatic)
Reagent Set

Expected Values[4]

Normal Range: Male (serum): 0.9 - 1.5 mg/dL
Male (urine): 1000 - 2000 mg/24hrs.
Female (serum): 0.7 - 1.4 mg/dL
Female (urine): 600 - 1500 mg/24hrs.

This range should serve only as a guideline. It is recommended that each laboratory establish its own range of expected values, since differences exist between instruments, laboratories and local populations.

Performance[5]

Data obtained on Hitachi 717

Correlation: Serum specimens (n = 30) were assayed by this method and by another commercial method. Statistical analysis revealed a correlation coefficient (r) of 0.9991, with a regression equation of y = 1.4815x - 0.5831.

Urine specimens (n = 37) were assayed by this method and by another commercial method. Statistical analysis revealed a correlation coefficient (r) of 0.9854, with a regression equation of y = 1.0545x + 0.3607.

Precision: (Performed according to NCCLS EP-5)

In Series (Intra Assay)

Mean (mg/dL)	SD	CV%
0.610	0.007	1.14
1.107	0.009	0.84
5.733	0.020	0.41

Day to Day (Inter Assay)

Mean (mg/dL)	SD	CV%
0.629	0.008	1.98
1.134	0.011	0.98
5.814	0.022	0.38

Sensitivity: Based on an instrument resolution of A = 0.001, the method presented shows a sensitivity of 0.04 mg/dL.

Linearity: (Peformed according to NCCLS EP6-P2) When performed as directed, it is linear to 30 mg/dL. Samples exceeding this value should be diluted 2-fold (1+1) with deionized water, the assay repeated and results multiplied by 2.

References

1. Pagana, KD and TJ Pagana, Mosby's Diagnostic and Laboratory Test Reference, 2nd Ed., Mosby, St. Louis, 1995, p.270.
2. Tietz, Norbert W, Clinical Guide to Laboratory Tests, 3rd Edition, WB Saunders, Philadelphia, 1995, pp 186-188.
3. Young DS et al. Clin Chem 21:286 D, 1975 (Special Issue)
4. Larsen K. Clin Chim Acta 41:209, 1972
5. Manufacturer's Laboratory Data

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