Creatinine

Enzymatic colorimetric

CE

Quantitative determination of creatinine concentration
Only for in vitro use in the clinical laboratory. Store at 2-8ºC .

REF: RCRL-100 / RCRL-200

Summary:

Creatinine measurements are used in the diagnosis and treatment of renal diseases, in monitoring renal dialysis, and as a calculation basis for measuring other urine analytes.
Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

Principle:

In the first reaction, creatinase and sarcosine oxidase are used in the enzymatic hydrolysis of endogenous creatine to produce hydrogen peroxide, directly eliminated by catalase. In the second reaction, the catalase is inhibited by sodium azide, and creatininase and 4-aminoantipyrine (4-AA) were added.The creatine generated from creatinine by creatininase is hydrolyzed sequentially by creatinase and sarcosine oxidase to produce hydrogen peroxide. This newly-formed hydrogen peroxide was measured in a coupled reaction catalyzed by peroxidase.

Composition:

R1: MOPS - 25 mmol/L, TOPS - 0.5 mmol/L, Creatinase - 10 KU/L, sarcosine oxidase - 5 KU/L, Catalase - 3 KU/L, EDTA - 1 mmol/L, pH 7.5
R2: MOPS - 90 mmol/L, Creatininase - 30 KU/L, peroxidase - 5 KU/L, sodium azide - 0.5 g/L, pH 7.5

Preparation:

R1 and R2 are ready to use.

Storage and Stability:

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC, protected from light and contaminations prevented during their use.
R1 and R2 are stable 8 weeks after opening bottle.

Samples:

Serum, plasma or urine. Dilute fresh urine 1/50 with distilled water before measurement.
Creatinine is stable 1 day at 2-8ºC.

Equipment:

- Spectrophotometer or photometer measuring at 545±20 nm, with cell holder thermostatable at 37ºC.
- General laboratory equipment.

Procedure:

1. Assay conditions:
Wavelength: . . . . . .546 nm
Temperature . . . . . .37°C (±0.1°C)
2. Bring the reagents and the instrument to 37ºC (±0.1ºC).
3. Pipette into a thermostatized cuvette:

	Blank	Calibrator	Sample
R1 (µL)	270	207	270
H2O/Cal/Sample (µL)	8	8	8

4. Mix and incubate 5 minutes
5. Read the absorbance (A1) of the calibrator and the samples, at 546 nm against the blank.
6: Add:

	Blank	Calibrator	Sample
R2 (µL)	90	90	90

7. Mix and incubate 5 minutes.
8. Read the absorbance (A2) of the calibrator and the sample, at 545 nm against the blank.

Calculations:

$$\frac{(A2s - A1s) - (A2b - A1b) \times K}{(A2s - A1s) - (A2b - A1b) \times K} \times \text{Concentration of Calibrator.} = \text{Creatinine}$$

K = 0.755 = 278 µL/368 µL

Quality control:

All clinical laboratories should establish an Internal Quality Control program. Check instrument and reagent performance with recommended controls or similar. The values obtained for QC should fall within manufacturer's acceptable ranges or should be established according to the Laboratory's QC program.
Controls should be assayed:
- Prior reporting patient results.
- Following any maintenance procedure on the photometer used.
- At pre-established intervals following the Q.C. Laboratory recommandations.

Reference values:

	Men	Women	
Serum or plasma	0.9 - 1.3	0.6 - 1.1	mg/dL
Urine	14 - 16	11 - 20	mg/Kg/24h

These values are for orientation purpose; each laboratory should establish its own reference range.

Performance characteristics:

Measuring range:
0.02-150mg/dL
Measuring with the operation method of the dosage and administration
Sensitivity testing:
1. The absorbance when making the purified water a sample, (A2b x A1b x k) is less than 0.050
2. The absorbance when making the standard solution of density 5mg/dL a sample, { (A2s x A1s x k) - (A2b x A1b x k)} is in the range of 0.120-0.180.
Singularity test:
When measuring a density serum for known management, known density± is within the 8%.
Reproducibility test:
When measuring an identical sample at the same time 10 times, coefficient of variance of the absorbance (CV%) is less than 5 %.
Accuracy:
Results obtained using these reagents did not show systematic differences when compared with other commercial reagents or with HPLC method. Details of the comparison experiments are available on request.
Interferences:
No interferences were observed with hemoglobin until 5 g/L, bilirubin 40 mg/dL. Other drugs and substances may interfere.

The results of the performance characteristics depend on the analyzer used.

Notes:

1. Calibration with an aqueous standard may cause matrix related bias, it is recommended to calibrate using a serum based calibrator.
2. Urine: multiply the result by 50 (sample dilution factor).

References:

1. Fossati et al. Clin Chem 1983; 29: 1494-1496.
2. Tietz Textbook of Clinical Chemistry, 3rd edition. Burtis CA, Ashwood ER. WB Saunders Co., 1999.
3. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
4. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.

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