

**Quantitative determination of creatinine
IVD**

Store at 2-8°C

PRINCIPLE OF THE METHOD

The assay is based on the reaction of creatinine with sodium picrate as described by Jaffé.

Creatinine reacts with alkaline picrate forming a red complex. The time interval chosen for measurements avoids interferences from other serum constituents.

The intensity of the color formed is proportional to the creatinine concentration in the sample¹.

CLINICAL SIGNIFICANCE

Creatinine is the result of the degradation of the creatine, component of muscles, it can be transformed into ATP, that is a source of high energy for the cells. The creatinine production depends on the modification of the muscular mass, and it varies little and the levels usually are very stable.

Is excreted by the kidneys. With progressive renal insufficiency there is retention in blood of urea, creatinine and uric acid.

Elevate creatinine level may be indicative of renal insufficiency^{1,4,5}.

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R 1 Picric Reagent	Picric acid	17,5 mmol/L
R 2 Alkaline Reagent	Sodium hydroxide	0,29 mol/L
CREATININE CAL	Creatinine aqueous primary standard 2 mg/dL	

PRECAUTIONS

R1/ R2: H314-Causes severe skin burns and eye damage.

CAL: H290-May be corrosive to metals.

Follow the precautionary statements given in MSDS and label of the product.

PREPARATION

Working reagent (WR):

Mix equal volumes of R 1 Picric Reagent and R 2 Alkaline reagent.

The working reagent is stable for 15 days at 2-8°C or 7 days at room temperature (15-25°C).

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use.

Do not use reagents over the expiration date.

Signs of reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 492 nm \geq 1,80.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 492 nm (490-510).
- Matched cuvettes 1,0 cm light path.
- General laboratory equipment.

SAMPLES

- Serum or heparinized plasma¹.

Creatinine stability: 24 hours at 2-8°C.

- Urine (24 h)¹: Dilute sample 1/50 with distilled water. Mix. Multiply results by 50 (dilution factor);

Creatinine stability: 7 days at 2-8°C.

PROCEDURE

1. Assay conditions:

Wavelength: 492 nm (490-510)

Cuvette: 1 cm. light path

Temperature: 37°C / 15-25°C

2. Adjust the instrument to zero with distilled water.

3. Pipette into a cuvette:

	Blank	Standard	Sample
WR (mL)	1,0	1,0	1,0
Standard ^(Note 1,2,3) (μL)	--	100	--
Sample (μL)	--	--	100

4. Mix and start stopwatch.

5. Read the absorbance (A₁) after 30 seconds and after 90 seconds (A₂) of the sample addition.

6. Calculate: $\Delta A = A_2 - A_1$.

CALCULATIONS

$$\frac{\Delta A \text{ Sample} - \Delta A \text{ Blank}}{\Delta A \text{ Standard} - \Delta A \text{ Blank}} \times 2 \text{ (Standard conc.)} = \text{mg/dL of creatinine in the sample}$$

Conversion factor: mg/dL x 88,4 = μmol/L.

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures: CONTROL Normal and Pathologic.

If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES¹

Serum or plasma:

Male 0,7 - 1,4 mg/dL \cong 61,8 - 123,7 μmol/L

Female 0,6 - 1,1 mg/dL \cong 53,0 - 97,2 μmol/L

Urine: 15-25 mg/Kg/24 h

Male 10 - 20 mg/Kg/24 h

Female 8 - 18 mg/Kg/24 h

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

PERFORMANCE CHARACTERISTICS

Measuring range: From detection limit of 0,000 mg/dL to linearity limit of 35 mg/dL.

If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dL)	0,92	3,43	0,96	3,50
SD	0,03	0,07	0,04	0,09
CV (%)	2,76	1,90	3,97	2,51

Sensitivity: 1 mg/dL = 0,0407 ΔAbs/min.

Accuracy: Results obtained using Audit Diagnostics reagents (y) did not show systematic differences when compared with other commercial reagents (x).

The results obtained using 50 samples were the following:

Correlation coefficient (r): 0,99584

Regression equation: $y = 0,953x + 0,075$

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

INTERFERENCES

Hemoglobin (1 g/L), Bilirubin (55 mg/dL), interfere¹.

A list of drugs and other interfering substances with creatinine determination has been reported^{2,3}.

NOTES

1. CREATININE CAL: Proceed carefully with this product because due its nature it can get contaminated easily.
2. Calibration with the aqueous Standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
3. Use clean disposable pipette tips for its dispensation.
4. **Audit Diagnostics has instruction sheets for several automatic analyzers.**

BIBLIOGRAPHY

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