Intended Use
For **IN VITRO quantitative** determination of Creatinine in serum or plasma using manual or automated applications.

Clinical Significance
Measurements of Creatinine are primarily used for diagnosing chronic hemorrhagic nephritis and impairment of kidney function, as well as for monitoring the causes and treatments. (1)

Method History
In 1886 Jaffe (2) described the reaction of Creatinine with picric acid in alkaline solution to form a red colored complex. Folin (3) applied the Jaffe reaction to the quantitative determination of Creatinine and creatine in urine. Several papers have appeared (4, 5) on the reaction mechanism between picric acid and Creatinine. The reaction is reported to be complex and dependent upon picric acid concentration, temperature and pH. Catachem's Creatinine method is based upon the Jaffe reaction and the work of Chasson, et al. (6)

Method Principle
The substance Creatinine present in the serum sample reacts at 37˚C with picric acid in alkaline solution to produce a red Tautomer complex which has a maximum absorption at 505nm. The reaction scheme illustrates the reaction that occurs in this method.

$$\text{Creatinine} + \text{Picric Acid} \xrightarrow[0H^-]{37^\circ C} \text{Creatinine-Picrate Complex}$$

Reagent Content
When reconstituted according to the directions, the concentration of the active ingredients in the reagents will be approximately as follows.

Creatinine Diluent
Each liter contains:

Water	
Sodium Hydroxide	0.5 mol/L
Surfactants and stabilizers	

Color Reagent
Each liter contains:

Water	
Picric Acid	22 mmol/L
Surfactants and stabilizers	

Precautions
Avoid contact of reagent with skin and eyes. Should contact occur, wash affected area with plenty of cold water. **DO NOT PIPETTE REAGENTS BY MOUTH.**

Preparation of Working Reagents
Prepare the required volume of Catachem Creatinine Working Reagent by mixing equal volumes of Creatinine Diluent and Creatinine Color Reagent. Store the Working Reagent in a closed container at room temperature.

Reagent Storage and Stability
Store all Creatinine Reagents at room temperature. When stored as directed, these reagents are stable until the expiration date stated on the label. When prepared and stored as directed, the Working Creatinine Reagent is stable for 21 days at room temperature.

Specimen Collection and Preparation
Test sera should be fresh, clear and unhemolyzed. When blood is drawn, it should be processed as soon as possible and the serum should be isolated from the clot without delay. In separated, unhemolyzed serum the creatinine concentration is stable for a few days if stored at 2-8˚C. To obtain longer stability, specimens should be frozen.

Quality Control
To monitor the performance of the Working Reagents and the procedure used we recommend the regular use of a normal and abnormal control.

Interfering Substances
Several substances have been reported to interfere with the Creatinine reaction. (7) Ascorbic acid and acetoacetate will significantly elevate the Creatinine values. A summary of the influence of drugs on clinical laboratory procedures may be found by consulting D.S. Young, et al. (8)

Expected Values
The range of expected values determined for this method is for humans is 0.7-1.5mg/dL. These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the species under test and for the area in which it is located.

Procedure
Important: Read the entire procedure instructions before proceeding with the assay.

Materials Required (Not Provided)

Spectrophotometer	
Match cuvettes	1 cm light path
Timer	to time incubation time
Pipette	2.0 ml for reagent
Pipette	0.1 ml for sample
Cylinder	25ml or 50ml for preparing Working Reagent

Materials Provided
Catachem Creatinine Diluent (R1) and Creatinine Color Reagent (R2).

Analytical Parameters

Wavelength	505nm
Temperature	37˚C
Pathlength	1 cm
Reaction Mode	rate, first order
Reaction Time	30 seconds
Reagent Volume	1.0 ml
Sample Volume	0.05 ml
Total Volume	1.05 ml
Sample-to-Reagent Ratio	1:21

Assay Procedure
1. Prepare the required volume of Creatinine Working Reagent by following instructions for Working Reagent preparation.
2. Set the spectrophotometer at 37˚C.
3. Pipette 1.0 ml of Creatinine Working Reagent into each of three cuvettes marked “Calibrator”, “Sample” and “Blank”.
4. Set the spectrophotometer wavelength at 505nm and zero the instrument with the “blank”.
5. Pipette 0.05 ml of Calibrator or Sample into their respective cuvettes. Mix all cuvettes well.
6. At exactly 20 seconds, read and record the absorbance (A1).
7. At exactly 30 seconds after the A1 reading, read again and record the absorbance (A2).
8. Calculate the Creatinine concentration (mg/dL) in the sample(s) as shown in calculations and results.

Calculations and Results

$$\text{Creatinine (mg/dL)} = \frac{\text{Sample (A2 - A1)}}{\text{Calibrator (A2 - A1)}} \times \text{Calibrator (mg/dL)}$$

Example:	A1	A2
Sample	0.01	0.03

Calibrator 0.01 0.05
Calibrator = 5.0 mg/dL

$$\text{Creatinine (mg/dL)} = \frac{0.03-0.01}{0.05-0.01} \times 5 \text{ mg/dL}$$

$$= 2.5 \text{ mg/dL}$$

Method Performance Characteristics
Sensitivity: The sensitivity of this method is 0.023-0.0286 absorbance unit per mg/dL.
Linear Range: There is no significant nonlinearity over the range of 0-20mg/dL.
Precision: Within-run and day-to-day precision is summarized below.

Creatinine Precision Study

Creat.	Within-Run Precision		Total Precision	
Mean	SD	CV	SD	CV
mg/dL	mg/dL	%	mg/dL	%
0.65	0.05	8.20	0.05	7.8
9.30	0.09	0.98	0.25	2.6
17.60	0.23	1.30	0.34	1.9

Correlation
A comparison of this method using an automated analyzer and a reference method based upon the Jaffe Reaction resulted in the following regression statistics.

Range	=	0.4-8.0 mg/dL
N	=	193
Y	=	1.000x - 0.04
r	=	0.996
Sy.x	=	0.16

References

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