ISE S.r.l. Creatinine – Instructions for use

CUSTOMISED SOLUTIONS FOR YOUR LABORATORY

CE IVD

R1: 2x 18 mL R2: 2x 4,5 mL **R3330000018** REF

Diagnostic reagent for quantitative in vitro determination of creatinine in human serum, plasma or urine on photometric systems

Test parameters

Method:	Colorimetric, 2 Point Kinetic, "mod. Jaffe", Increasing reaction
Wavelength:	Hg 492 nm (490 nm - 510 nm)
Temperature:	20 °C – 25 °C / 37 °C
Sample:	Serum, heparin plasma, urine
Linearity:	up to 15 mg/dL (1330 µmol/L)
Sensitivity:	Lower limit of detection: 0.2 mg/dL (17.7 µmol/L)

Summary [1,2]

Creatinine is a waste product excreted by the kidneys mainly by glomerular filtration. The concentration of creatinine in plasma of a healthy individual is fairly constant, independent from water intake, exercise and rate of urine production. Therefore, increased plasma creatinine values always indicate decreased excretion, i.e. impaired kidney function. The creatinine clearance enables a quite good estimation of the glomerular filtration rate (GFR) which allows better detection of kidney diseases and monitoring of renal function. For this purpose creatinine is measured simultaneously in serum and urine collected over a defined time period.

TEST PRINCIPLE

Creatinine forms a coloured orange-red complex in an alkaline picrate solution. The difference in absorbance at fixed times during conversion is proportional to the concentration of creatinine in the sample.

REAGENT COMPOSITION

	COMPONENTS	CONCENTRATIONS
R1:	Sodium Hydroxide	0.2 mol/L
R2:	Picric Acid	20 mmol/L

REAGENT PREPARATION

Substrate Start
The reagents are ready to use.
Sample Start:
Mix 4 parts of R1 with 1 part of R2 (= Working Reagent)

REAGENT STABILITY AND STORAGE

Conditions:	Protect from direct light. Close immediately after use. Do not freeze the reagents.	
Stability:	at 2 – 25 °C	up to the expiration date
Sample Start (Working Reagent):		
Stability:	at 15 – 25 °C	5 hours

sample PREPARATION
Urine: Dilute urine 1 + 49 with dist. water. Multiply result by 50. (The urine controls Diacon Urine must be prediluted in the same way as patient samples.)

SAMPLE STABILITY AND STORAGE [5]

	at 4 – 25 °C	7 days
serum/heparin plasma:		
	at -20 °C	at least 3 months
urine:	at 20 – 25 °C	2 days
	at 4 – 8 °C	6 days
	at -20 °C	6 months

Freeze only once. Discard contaminated specimens.

Standard

(has to be ordered separately)

Concentration:	2 mg/dL (177 µmol/L)
Storage:	2 – 25 °C
Stability:	up to the expiration date

Close immediately after use!

Materials required but not provided

NaCl solution (9 g/L)
General laboratory equipment

MANUAL TEST PROCEDURE

Bring reagents and samples to room temperature.

Substrate Start

Pipette into test tubes	Blank	Std/Cal.	Sample
Reagent 1	1000 µl	1000 µL	1000 µL
Sample	-	-	50 µL
Std./Cal.	-	50 µL	-
Dist. water	50 µL	-	-
Mix. Incubate 0 - 5 min., then add:			
Reagent 2	250 µL	250 µL	250 µL
Mix. Incubate for exactly 1 min. and read A1against reagent blank. Incubate for exactly 2 min. and read A2 against reagent blank. Calculate: ΔA = (A2 - A1) sample or standard			

Sample Start

Pipette into test tubes	Blank	Std/Cal.	Sample
Working Reagent	1000 µL	1000 µL	1000 µL
Sample	-	-	50 µL
Std./Cal.	-	50 µL	-
Dist. water	50 µL	-	-
Mix. Incubate for exactly 1 min. and read A1 against reagent blank. Incubate for exactly 2 min. and read A2 against reagent blank. Calculate: ΔA = (A2 - A1) sample or standard			

CALCULATION

Serum/Plasma:

$$\text{Creatinine [mg/dL]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Std/Cal}} \times \text{Conc. Std/Cal [mg/dL]}$$

Urine:

$$\text{Creatinine [mg/dL]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Std/Cal}} \times \text{Conc. Std/Cal [mg/dl]} \times 50$$

Creatinine Clearance [7]

$$\text{[mL/min/1.73 m}^2\text{]} = \frac{\text{mg Creatinine/100 ml Urine x ml Urine}}{\text{mg Creatinine/100 ml Serum x urine collection time}}$$

The calculated creatinine clearance refers to the average body surface of an adult (1.73 m^2).

unit conversion

mg/dL x 88.4 = µmol/L

compensated method [3,4]

Picric acid which forms the coloured complex reacts unspecifically with interfering serum components, so-called pseudo-creatinines. This leads to falsely elevated creatinine values in serum and plasma samples especially in the low measuring range. To compensate these interferences the calibrator value for the compensated method indicated in the value sheet of our calibrator has to be used for calculation. Additionally 0.3 mg/dL (27 µmol/L) has to be subtracted from the calculated creatinine value. For use of the compensated method calibration with the calibrator our calibrator is strictly recommended. The method is applicable only for serum and plasma samples.
The compensated method is traceable to GC-IDMS.

REFERENCE RANGE*
Serum / Plasma, not compensated:

Adults [1]	mg/dL	µmol/L
Women	0.6 – 1.1	53 – 97
Men	0.7 – 1.3	62 – 115
Children [2,8]		
Neonate	0.5 – 1.2	44 – 106
Infant	0.4 – 0.7	35 – 62
Child	0.5 – 1.2	44 – 106

Serum / Plasma, compensated:

Adults [3]	mg/dL	µmol/L
Women	0.5 – 0.9	44 – 80
Men	0.7 – 1.2	62– 106
Children [9]		
Neonate	0.24 – 1.04	21 – 92
Infant	0.17 – 0.42	15 – 37
Child	0.24 – 0.87	21 – 77

24h Urine [1]:

Women	11 – 20 mg/kg/24h	97 – 177 µmol/kg/24h

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Men	14 – 26 mg/kg/24h	124 – 230 µmol/kg/24h

Creatinine clearance [2]:

Women	95 - 160 mL/min/1.73 m^2
Men	98 - 156 mL/min/1.73 m^2

* Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

PERFORMANCE CHARACTERISTICS

LINEARITY/MEASURING RANGE

The test has been developed to determine creatinine concentrations within a measuring range from 0.2 – 15 mg/dL (18 – 1330 µmol/L). Above this concentration, samples should be diluted 1 + 1 with NaCl solution (9 g/L in dist. water) and re-assayed multiplying the result by 2.

SENSITIVITY/LIMIT OF DETECTION
The lower limit of detection is 0.2 mg/dL (17.7 µmol/L)

PRECISION (at 37°C)

Intra-assay, n = 20	Mean [mg/dl]	SD [mg/dl]	CV [%]
Sample 1	0.56	0.01	1.30
Sample 2	1.24	0.01	0.83
Sample 3	6.73	0.06	0.93
Inter-assay, n = 20	Mean [mg/dl]	SD [mg/dl]	CV [%]
Sample 1	0.81	0.03	3.63
Sample 2	1.60	0.01	0.87
Sample 3	5.73	0.05	0.85

SPECIFICITY/INTERFERENCES

no interference up to:	Ascorbic acid	30 mg/dL
	Bilirubin	4 mg/dL
	Hemoglobin	500 mg/dL
	Triglycerides	2000 mg/dL

For further information on interfering substances refer to Young DS [10].

METHOD COMPARISON

A comparison of this Creatinine (y) with a commercially available Jaffé method (x) using 68 human sera samples within a range of 0.6 – 10 mg/dL (53.0 – 884 µmol/L) gave following results:
y = 1.014 x – 0.031 mg/dL; r = 1.000.
A comparison of our Creatinine compensated (y) with the enzymatic method (x) using 65 human sera samples within a range of 0.5 – 4.3 mg/dL (44.2 – 380 µmol/L) gave following results:
y = 0.986 x + 0.043 mg/dL; r = 0.998.

Calibration

The assay requires the use of a creatinine standard or calibrator.
We recommend our calibrator.

Calibrator values have been made traceable to NIST (National Institute for Standardization) Standard Reference Material SRM 967 using level 1 and 2 and therefore to GC-IDMS (gas chromatography – isotope dilution mass spectrometry).

QUALITY CONTROL

All controls with Creatinine values determined by this method can be used. We recommend our serum controls Normal (control serum with values in the normal range) and Pathological (control serum with values in the abnormal range).
Each laboratory should establish corrective action in case of deviations in control recovery.

WARNINGS AND PRECAUTIONS

1. Reagent 1: Warning. H290: May be corrosive to metals. H315: Causes skin irritation. H319: Causes serious eye irritation. P234: Keep only in original container. P264: Wash hands and face thoroughly after handling. P280: Wear protective gloves/protective clothing/eye protection/face protection. P302+P352: If on skin: Wash with plenty of water/soap. P332+P313: If skin irritation occurs: get medical advice/attention. P305+P351+P338: If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P337+P313: If eye irritation persists: Get medical advice/attention. P390: Absorb spillage to prevent material damage.
2. Reagent 2: Warning. H290: May be corrosive to metals. P234: Keep only in original container. P280: Wear protective gloves/protective clothing/eye protection/face protection. P390: Absorb spillage to prevent material damage.
3. High homogentisic acid concentrations in urine samples lead to false results.
4. In very rare cases, samples of patients with gammopathy might give falsified results [11].
5. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.
6. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
7. For professional use only!

WASTE MANAGEMENT

Please refer to local requirements.

References

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Symbols on labels and packaging

IVD = In vitro diagnostic medical device

REF = Catalog Number

LOT = Lot Number

= Manufacturer

= Expiration date

= Temperature limitation

= Instruction for use

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