DIAGNOSTICA PRODUCTS

Liquid Reagents – ready to use

CREATININE
Enzymatic, PAP

2 Reagents

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

Ref.No.	Kit Size	Content
DIA010090	40 ml	2x15 ml R1 + 1x10 ml R2
DIA010092	4 x 20 ml	3x20 ml R1 + 1x20 ml R2
DIA010093	4 x 25 ml	3x25 ml R1 + 1x25 ml R2

Additionally offered:

DIA040012	1 x 3 mL	**Diacal Auto** (Calibrator)
DIA030012	1 x 5 mL	**Diacon N** (Control Normal)
DIA030022	1 x 5 mL	**Diacon P**(Control Abnormal)
DIA030030	1 x 5 mL	**Diacon Urine Level 1** (Control Normal)
DIA030035	1 x 5 mL	**Diacon Urine Level 2** (Control Abnormal)

TEST PARAMETERS

Method: Colorimetric, enzymatic, endpoint, increasing reaction
Wavelength: 550 nm
Temperature: 37°C
Sample: Serum, urine
Linearity: up to 30 mg/dL(2650mmol/L)
Sensitivity: Thelimit of detection is 0.14 mg/dL (12 mmol/L).

SUMMARY [1,2]

Creatinine is a chemical waste molecule that is generated from muscle metabolism. Creatinine is produced from creatine, a molecule of major importance for energy production in muscles. Approximately 2 % of the body's creatine is converted to creatinine every day. Creatinine is transported through the bloodstream to the kidneys. The kidneys filter out most of the creatinine and dispose of it in the urine. The kidneys maintain the blood creatinine in a normal range. Creatinine has been found to be a fairly reliable indicator of kidney function. As the kidneys become impaired the creatinine level in the blood will rise. Abnormally high levels of creatinine thus warn of possible malfunction or failure of the kidneys, sometimes even before a patient reports any symptoms. It is for this reason that standard blood and urine tests routinely check the amount of creatinine in the blood.

TEST PRINCIPLE

The enzymatic assay for creatinine involves a series of coupled enzymatic reactions including creatininase enzymatic conversion of creatinine into the product creatine which itself is converted to sarcosine by creatinase, followed by oxidation of sarcosine by sarcosine oxidase (SOD) producing hydrogen perox.
In the presence of peroxidase (POD) the hydrogen peroxide is quantified at 550 nm by the formation of a colored dye[4].

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

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

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

$$2\,H_2O_2 + \text{4-AA} + \text{TOOS} \xrightarrow{\text{Peroxidase}} \text{quinone dye}$$

The absorbance of the produced red dye is proportional to the creatinine concentration in the sample.
Any endogenous creatine present in the sample is removed by creatinase and sarcosine oxidase during preincubation.

REAGENT COMPOSITION

COMPONENTS	CONCENTRATION	
Reagent1: (R1)		
Good's Buffer, pH 7-8		
Creatinase	12 – 60	kU/L
Sarcosine oxidase (SOD)	4 – 17	kU/L
TOOS	0.07 – 0.21	g/L
Ascorbate oxidase		
Reagent 2: (R2)		
Good's Buffer, pH 7-8		
Creatininase	135 – 670	kU/L
Peroxidase		
4-Aminoantipyrine (4-AA)	0.3 – 0.9	g/L

REAGENT PREPARATION

Substrate Start
The reagents are ready to use.
Sample Start:
Not possible (elimination of endogenous creatine).

REAGENT STABILITY AND STORAGE

Conditions: Reagents are light-sensitive. →
Protect from light!
Close immediately after use.
Do not freeze the reagents!
Avoid contamination!
Storage: at 2 – 8°C
Stability (unopened): up to theindicated expiration date
On board stability: 30 days

SAMPLE PREPARATION

Urine: Dilute urine 1 + 9 with distilled water. [Diacon Urine controls must be diluted in the same way as patient urine samples.]

SAMPLE STABILITY AND STORAGE [7]

serum:	at 4 – 25 °C	7 days
	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.

MATERIALS REQUIRED BUT NOT PROVIDED

NaCl solution (9 g/L)
General laboratory equipment

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! Avoid contamination!

MANUAL TEST PROCEDURE

Bring reagents and samples to room temperature.

Pipette into test tubes	Blank	Std/Cal.	Sample
Reagent 1	900 µL	900 µL	900 µL
Sample	-	-	20 µL
Stdandard/Calibrator	-	20 µL	-
Dist. water	20 µL	-	-
Mix. Incubate 5 min. at 37°C and read absorbance A1at 550 nm against the blank.Then add:			
Reagent 2	300 µL	300 µL	300 µL
Mix. Incubate 5 min. at 37°C and read absorbance A2 at 550 nm against the blank. Calc.: ΔA = (A2 – 0.754 A1) sample or standard.			

CALCULATION

Serum :

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

Urine :

$$\text{Creatinine [mg/dL]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Std/Cal}} \times \text{Conc. Std/Cal [mg/dL]} \times 10$$

DIAGNOSTICA PRODUCTS, RTE DE GABES KM 9 Z.I THYNA AL JADIDA SFAX, TUNISIA.
Tel. +216 74 68 79 40 Fax. +216 74 68 79 32

REV. 01, 24.11.2017

UNIT CONVERSION

mg/dL x 88.4 = µmol/L

REFERENCE RANGE [6] *

Serum:

	mg/dL	µmol/L
Females	0.51 – 0.95	45 – 84
Males	0.67 – 1.17	59 – 104

First morning urine:

	mg/dL	µmol/L
Females	29 – 226	2550 – 20000
Males	40 – 278	3540 – 24600

* These values are for orientation purpose.Each laboratory should check if reference ranges are transferable to its own patient population and determine own reference ranges as necessary.

PERFORMANCE CHARACTERISTICS

LINEARITY, MEASURING RANGE

The test has been developed to determine creatinine concentrations within a measuring range from 0.14 – 30 mg/dL.
If values exceed this range samples should be diluted with NaCl solution (9 g/L) and the result multiplied by the dilution factor.

SENSITIVITY/LIMIT OF DETECTION

The lower limit of detection is 0.14 mg/dL (12 µmol/L)

PRECISION (at 37°C)

Serum Testing

Within run precision	Mean	SD	CV
n = 80	[mg/dL]	[mg/dL]	[%]
Sample 1	0.74	0.015	2.1
Sample 2	1.38	0.015	1.1
Sample 3	4.04	0.029	0.7

Total precision	Mean	SD	CV
n = 80	[mg/dL]	[mg/dL]	[%]
Sample 1	0.74	0.022	3.0
Sample 2	1.38	0.026	1.9
Sample 3	4.04	0.058	1.4

Urine Testing

Within run precision	Mean	SD	CV
n = 21	[mg/dL]	[mg/dL]	[%]
Sample 1	29.09	0.10	0.36
Sample 2	87.1	0.27	0.31
Sample 3	196.7	0.90	0.46

Total precision	Mean	SD	CV
n = 20	[mg/dL]	[mg/dL]	[%]
Sample 1	29.9	0.79	2.64
Sample 2	87.7	0.67	0.76
Sample 3	195	1.19	0.60

SPECIFICITY/INTERFERENCES

No interference up to:

AscorbicAcid	10 mM
Bilirubin	40 mg/dL
Bilirubin, conjugated	30 mg/dL
Hemoglobin	500 mg/dL
Triglycerides	1000 mg/dL

For further information on interfering substances refer to Young DS [8].

METHOD COMPARISON

This assay (y) was compared with a legally marketed creatinine assay (x) using serum samples ranging from 0.2 to 13.51 mg/dL(17.7 – 1194 µmol/L) and urine samples ranging from 0.14 to 141 mg/dL (12.4 – 12434 µmol/L):
Serum samples: y = 0.9467 x + 0.0643; r= 0.9981
Urine samples: y = 1.0002 x – 0.0518; r = 0.9968

CALIBRATION

The assay requires the use of a Creatinine Standard or Calibrator. We recommend the Diagnostica **Creatinine Standard** and the DIAGNOSTICA multi calibration serum **Diacal Auto**.
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).
NOTE: calibration of serum samples with an aqueous standard may cause matrix related bias. It is recommended to calibrate serum samples with a serum based calibrator.

QUALITY CONTROL

All control sera and urine controls with Creatinine values determined by this method can be used.
We recommend the DIAGNOSTICA serum controls **Diacon N** (control serum with values in the normal range) and **Diacon P** (control serum with values in the abnormal range) as well as the Diagnostica urine controls **Diacon Urine Level 1** (control urine normal) and **Level 2** (control urine abnormal).
Each laboratory should establish corrective action in case of deviations in control recovery.

AUTOMATION

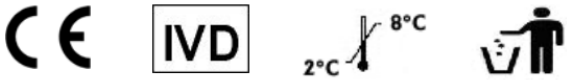
Special applications for automated analyzers can be made on request.

WARNINGS AND PRECAUTIONS

1. The reagent contains sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. N-acetylcysteine (NAC), acetaminophen and metamizole medication leads to falsely low results in patient samples.
3. Please refer to the safety data sheet and take the necessary precautions for the use of laboratory reagents.
4. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
5. For professional use only!

WASTE MANAGEMENT

Please refer to local legal requirements.



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