Creatinine PAP FS*

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

Order information

Cat. No.	Kit size						
1 1759 99 10 021	R1	4 x	20 mL	+	R2	2 x	20 mL
1 1759 99 10 026	R1	4 x	100 mL	+	R2	2 x	100 mL
1 1759 99 10 023	R1	1 x	800 mL	+	R2	1 x	400 mL
1 1759 99 10 704	R1	8 x	40 mL	+	R2	8 x	20 mL
1 1759 99 10 917	R1	8 x	30 mL	+	R2	8 x	15 mL

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 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.

Method

Enzymatic colorimetric test

Principle

Creatinine is determined by the following reaction:

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

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

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

$$H_2O_2 + \text{HTIB} + \text{4-AA} \xrightarrow{\text{Peroxidase}} \text{Quinone dye}$$

The absorbance of the produced red dye at 545 nm is proportional to the creatinine concentration in the sample.

Reagents

Components and Concentrations

R1:	Goods Buffer	pH 8.1	25 mmol/L
	Creatinase		≥ 30 kU/L
	Sarcosine oxidase		≥ 10 kU/L
	Ascorbate oxidase		≥ 2.5 kU/L
	Catalase		≥ 350 kU/L
	HTIB (3-Hydroxy 2,4,6-triiodo benzoic acid)		2.3 mmol/L
R2:	Goods Buffer	pH 8.1	25 mmol/L
	Creatininase		≥ 150 kU/L
	Peroxidase		≥ 50 kU/L
	4-Aminoantipyrine (4-AA)		2 mmol/L
	Potassium hexacyanoferrate		0.18 mmol/L

Storage Instructions and Reagent Stability

The reagents are stable up to the end of the indicated month of expiry, if the reagents are stored at 2 – 8°C and contamination is avoided. Do not freeze the reagents and protect them from light!

Warnings and Precautions

1. Reagent 2 contains sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. Some clinical chemistry reagents may cause interferences. Please take care to avoid contamination and carry-over. Special caution is needed when using reagents for the measurement of HDL-C and LDL-C. Consumables have to be cleaned thoroughly after use with other tests. I case of automated measurements please refer to the system manual for special programs.
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 [9.]
5. N-acetylcysteine (NAC), acetaminophen, metamizole and phenindione medication leads to falsely low, eltrombopag medication to falsely low or high results in patient samples.
6. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. 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 legal requirements.

Reagent Preparation

Reagents are ready to use.

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Specimen

Serum, heparin plasma, urine
Stability [4]

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

Dilute urine 1 + 9 with dist. water; multiply the result by 10. TruLab urine controls must be prediluted the same way as patient samples.

Discard contaminated specimens! Only freeze once!

Assay Procedure

Application sheets for automated systems are available on request.

Wavelength	Hg 546 nm
Optical path	1 cm
Temperature	37°C
Measurement	Against reagent blank

	Blank	Sample/Calibrator
Sample/Calibrator	-	24 µL
Dist. water	24 µL	-
Reagent 1	1000 µL	1000 µL
Mix, incubate 5 min. and read absorbance A1, then add:		
Reagent 2	500 µL	500 µL
Mix and read absorbance A2 after 5 min.		

$\Delta A = (A2 - 0.672\ A1)$ Sample/Calibrator

Calculation

With calibrator

Serum/plasma

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

Urine

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

* fluid stable

Creatinine-Clearance [mL/min/1,73 m^2] [6]

$$= \frac{\text{mg Creatinine / 100 mL Urine} \times \text{mL Urine}}{\text{mg Creatinine / 100 mL Serum} \times \text{min Urine collection time}}$$

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

Conversion factor

Creatinine [mg/dL] x 88.4 = Creatinine [µmol/L]

Calibrators and Controls

For the calibration of automated photometric systems, DiaSys TruCal U calibrator is recommended. The 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). Creatinine Standard FS may be used alternatively for calibration. For internal quality control DiaSys TruLab N, P and TruLab Urine controls should be assayed. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
TruCal U	5 9100 99 10 063	20 x 3 mL
	5 9100 99 10 064	6 x 3 mL
TruLab N	5 9000 99 10 062	20 x 5 mL
	5 9000 99 10 061	6 x 5 mL
TruLab P	5 9050 99 10 062	20 x 5 mL
	5 9050 99 10 061	6 x 5 mL
TruLab Urin Level 1	5 9170 99 10 062	20 x 5 mL
	5 9170 99 10 061	6 x 5 mL
TruLab Urin Level 2	5 9180 99 10 062	20 x 5 mL
	5 9180 99 10 061	6 x 5 mL
Creatinine Standard FS	1 1700 99 10 030	6 x 3 mL

Performance Characteristics

Measuring range

The test has been developed to determine creatinine concentrations within a measuring range from 0.03 – 160 mg/dL (2.65 – 14144 µmol/L). The upper limit of the measuring range at the same time depends on the photometer linearity of the analyzer and may vary. When values exceed this range, samples should be diluted 1 + 1 with NaCl solution (9 d/L) and the result multiplied by 2.

Specificity/Interferences

No interference was observed by ascorbic acid up to 25 mg/dL, bilirubin up to 20 mg/dL, hemoglobin up to 400 mg/dL, creatine up to 40 mg/dL and lipemia up to 1500 mg/dL triglycerides. Proline in concentrations > 12 mg/dL leads to falsely elevated values. For further information on interfering substances refer to Young DS [8].

Sensitivity/Limit of Detection

The lower limit of detection is 0.03 mg/dL (2.65 µmol/L).

Precision

Intra-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	0.53	0.01	1.92
Sample 2	1.33	0.02	1.27
Sample 3	8.79	0.04	0.49

Inter assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	0.53	0.02	4.02
Sample 2	1.10	0.03	3.00
Sample 3	8.49	0.14	1.63

Method Comparison

A comparison of DiaSys Creatinine PAP FS (y) with a commercially available test (x) using 102 serum and plasma samples within a range of 0.4 – 18 mg/dL (35 - 1591 µmol/L) gave following results:
y = 1.02 x – 0.02 mg/dL; r = 1.00.

A comparison of DiaSys Creatinine PAP FS (y) with a commercially available test (x) using 29 urine samples within a range of 1.4 – 27 mg/dL (124 – 2387 µmol/L) gave following results:
y = 1.051 x – 0.08 mg/dL; r = 1.00.

Reference Range

Serum/Plasma

	mg/dL	µmol/L
Adults [3]		
Women	0.51 – 0.95	45 – 84
Men	0.67 – 1.17	59 – 104
Children [7]		
0 – 7 days	0.6 – 1.1	53 – 97
1 week – 1 month	0.3 – 0.7	27 – 62
1 – 6 month(s)	0.2 – 0.4	18 – 35
7 – 12 months	0.2 – 0.4	18 – 35
1 – 18 year(s)	0.2 – 0.7	18 – 62

1st Morning urine [3]

Women	29 – 226 mg/dL	2.55 – 20.0 mmol/L
Men	40 – 278 mg/dL	3.54 – 24.6 mmol/L

24h urine [6]

Women	720 – 1510 mg/24h	6 – 13 mmol/24h
Men	980 – 2200 mg/24h	9 – 19 mmol/24h

Albumin/creatinine ratio (early morning urine) [10]:
< 30 mg/g Creatinine

Creatinine clearance [6]

66.3 – 143 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.

Literature

1. Newman DJ, Price CP. Renal function and nitrogen metabolites. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 1204-1270.
2. Thomas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p. 366-74.
3. Mazzachi BC, Peake M, Erhardt V. Reference range and method comparison for enzymatic and Jaffé Creatinine assays in plasma and serum and early morning urine. Clin Lab 2000; 46: 53-5.
4. Guder WG, Zawta B. Recommendations of the Working group on Preanalytical Quality of the German Society for Clinical Chemistry and the German Society for Laboratory Medicine: The Quality of Diagnostic Samples. 1st ed Darmstadt: GIT Verlag 2001; p. 24-5,50-1.
5. Levey AS, Coresh J, Greene T, Marsh J et al: Expressing the Modification of Diet in Renal Disease Study Equation for Estimating Glomerular Filtration Rate with Standardized Serum Creatinine Values. Clin Chem 2007; 53 (4): 766-72.
6. Junge W, Wilke B, Halabi A, Klein G. Determination of reference intervals for serum creatinine, creatinine excretion and creatinine clearance with an enzymatic and a modified Jaffé method. Clin Chim Acta 2004; 344: 137-148.
7. Soldin SJ, HicksJM. Pediatric reference ranges . Washington: AACC Press, 1995:50.
8. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th. ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
9. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007;45(9):1240-1243.
10. Dati F, Metzmann E. Proteins-Laboratory testing and clinical use. 1st ed. Holzheim: DiaSys Diagnostic Systems; 2005: p. 93.

Manufacturer

IVD CE

DiaSys Diagnostic Systems GmbH
Alte Strasse 9 65558 Holzheim Germany