Creatinine Reagent - Enzymatic
2 Part Liquid

PRODUCT SUMMARY

Stability	:	Until Expiry at 2-8°C
Measuring Range	:	0.2-150 mg/dL (18-13260 µmol/L)
Specimen Type	:	Serum, plasma or urine
Method	:	Endpoint
Reagent Preparation	:	Supplied ready to use.

IVD

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorized Representative		Temperature Limitation
IVD	For in vitro diagnostic use		Use by/Expiration Date
LOT	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
REF	Catalogue number		Manufactured by
	Consult instructions for use	REAG 2	Reagent 2 (R2)
REAG 1	Reagent 1 (R1)		

INTENDED USE
This reagent is intended for the in vitro quantitative determination of Creatinine in human serum, plasma or urine.

CLINICAL SIGNIFICANCE [1,2]
Creatinine is a de-composition by product of the energy producing compound, creatine phosphate. The amount of creatinine produced is fairly constant and is primarily a function of muscle mass. Creatinine is removed from the plasma by glomerular filtration and then excreted in the urine without any appreciable re-absorption by the tubules. Typically 7-10 % of creatinine in the urine is derived from tubular secretion but this is increased in the presence of renal insufficiency.
Because creatinine is endogenous and is freely filtered at the glomerulus, it is widely used to assess kidney function (Glomerular Filtration Rate or GFR) and is expressed either as a plasma concentration or renal clearance. Elevated levels of plasma creatinine are associated with impaired renal function. However, as serum creatinine is affected by factors independent of GFR including tubular secretion, age, sex, body size, diet, certain drugs and methodology, a normal plasma creatinine does not necessarily equate with normal kidney function. Therefore, serum creatinine alone should not be used to estimate GFR or detect the presence of impaired renal function.
More accurate and precise estimations of GFR can be obtained with equations that are designed to average the effects of factors that affect serum creatinine other than GFR. One such equation was developed as a result of the Modification of Diet in Renal Disease (MDRD) study, however, these formulas too have there limitations, especially in patients with acute renal failure and children, and do not take into account variations in assay specificity and calibration. Nevertheless, the use of an equation such as the MDRD study equation is recommended above the use of serum creatinine alone.

METHODOLOGY
The method employed here is based on an enzymatic colorimetric determination of creatinine which largely eliminates interferences known to the Jaffe method. The series of enzyme catalyzed reactions involved in the assay system are as follows:

$$\text{Creatinine} + H_2O \xrightarrow{\textit{Creatinine amidohydrolase}} \text{Creatine}$$

$$\text{Creatine} + H_2O \xrightarrow{\textit{Creatine amidinohydrolase}} \text{Sarcosine} + \text{Urea}$$

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

$$2H_2O_2 + \text{4-aminoantipyrine} + \text{TOPS}^* \xrightarrow{\textit{Peroxidase}} \text{Quinoneimine Dye} + 4H_2O$$

* TOPS: N-Ethyl-N-sulfopropyl-m-toluidine

In the final reaction sequence, the formation of the quinoneimine dye product results in an increase in absorbance at 550 nm (530-570 nm) which is directly proportional to the creatinine concentration in the sample. Potential interference from endogenous creatine and sarcosine are eliminated by the reaction of creatine amidinohydrolase, sarcosine oxidase and catalase before creatinine is determined. Ascorbate oxidase is included in the reagent to eliminate the influence of ascorbate in the sample.

REAGENT COMPOSITION

Active Ingredients	Concentration
Reagent 1 (R1)	
Buffer	25 mmol/L
Creatine amidinohydrolase	25000 U/L
Sarcosine oxidase	8000 U/L
Ascorbate oxidase	5000 U/L
TOPS (N-Ethyl-n-sulfopropyl-m-toluidine)	0.47 mmol/L
Reagent 2 (R2)	
Buffer	100 mmol/L
Creatinine amidohydrolase	300000 U/L
Peroxidase	10000 U/L
4-Aminoantipyrine	3 mmol/L

pH 7.5 ± 0.1 at 20°C

WARNING: Do not ingest. Avoid contact with skin and eyes. If spilt, thoroughly wash affected areas with water. Reagent contains sodium azide which may react with copper or lead plumbing. Flush with plenty of water when disposing. For further information, consult the Creatinine Reagent - Enzymatic Material Data Safety Sheet.

REAGENT PREPARATION
The reagents are supplied ready to use.

STABILITY AND STORAGE
When stored capped at 2-8°C, the reagents are stable until the expiration date stated on the bottle and kit box labels.
Indications of Reagent Deterioration:
- Turbidity;
- Reagent 1 Absorbance >1.0 AU (550 nm, 1cm lightpath);
- Reagent 2 Absorbance >0.15 AU (550 nm, 1cm lightpath); and/or
- Failure to recover control values within the assigned range

SPECIMEN COLLECTION AND HANDLING
Serum: Use non-haemolysed serum.
Plasma: Use EDTA plasma.
Urine: Collect Urine without preservatives. For analyzers which do not have automatic dilution, urine samples should be prediluted 1:10 with distilled or deionized water. Multiply results by the dilution factor.
Storage: Serum/Plasma Creatinine samples are stable for up to 2 weeks at 4°C and up to 42 days when stored frozen[3]. Urine Creatinine samples are stable for up to 3 days at 4°C.[4]

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED
- A clinical chemistry analyzer capable of maintaining constant temperature (37°C) and measuring absorbance at 550 nm (530-570 nm).
- If required, pipettes for accurately dispensing measured volumes.
- Analyzer specific consumables, eg: sample cups.
- Normal and Abnormal assayed control material.
- Calibrator or a suitable aqueous Creatinine standard.

ASSAY PROCEDURE
The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

SYSTEM PARAMETERS

Temperature	37°C
Primary Wavelength	550 nm (530-570 nm)
Assay Type	Endpoint
Direction	Increase
Sample : Reagent 1 : Reagent 2 Ratio	1 : 45(R1) : 15(R2)
eg. Sample Volume	6 µL
Reagent 1 Volume	270 µL
Reagent 2 Volume	90 µL
Incubation Time (Sample + R1)	5 minutes
Read Time (Sample + R1 + R2)	5 minutes
Reagent Blank Limits (R1 + R2)	Low 0.0 AU
(550 nm, 1cm light path)	High 0.2 AU
Measuring Range	0.2 - 150 mg/dL
	(18 - 13260 µmol/L)
Analytical Sensitivity	21.4 ΔmAbs per mg/dL
(546 nm, 1cm light path)	(0.242 ΔmAbs per µmol/L)

CALCULATIONS
Results are calculated, usually automatically by the instrument, as follows:

$$\text{Creatinine} = \frac{\Delta\text{Abs of Unknown}}{\Delta\text{Abs of Calibrator}} \times \text{Calibrator Value}$$

Example:
Absorbance of calibrator = 0.039
Absorbance of unknown = 0.021
Value of calibrator = 2.8 mg/dL (252 µmol/L)

$$\text{Creatinine} = \frac{0.021}{0.039} \times 2.8 = 1.5 \text{ mg/dL}$$

$$\text{Creatinine} = \frac{0.021}{0.039} \times 252 = 136 \text{ µmol/L}$$

For urine specimens the results must be multiplied by the dilution factor and 24 hour collections by the volume in liters.

$$\text{Urine Creatinine (g/24 hours)} = \frac{\text{Creatinine Result (mg/dL)}}{100} \times \text{Dilution Factor} \times \text{Volume (L)}$$

$$\text{Urine Creatinine (mmol/24 hours)} = \frac{\text{Creatinine Result (µmol/L)}}{1000} \times \text{Dilution Factor} \times \text{Volume (L)}$$

Example:
Creatinine result = 14.0 mg/dL (1240 µmol/L)
Dilution of Urine = 11 (1mL Urine + 10 mL H_2O)
24 Hour volume of urine = 0.95 Liters

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Urine Creatinine = 14.0/100 x 11 x 0.95 = 1.5 g/24 hours
Urine Creatinine = 1240/1000 x 11 x 0.95 = 13 mmol/24 hours

NOTES
1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer or analyzer requirements.
2. Unit conversion: µmol/L x 0.0113 = mg/dL.

CALIBRATION
Calibration is required. An aqueous standard or serum based calibrator, with an assigned value traceable to a primary standard (eg NIST or IRMM) is recommended. For calibration frequency on automated instruments, refer to the instrument manufacturers specifications.
However, calibration stability is contingent upon optimum instrument performance and the use of reagents which have been stored as recommended in the stability and storage section of this package insert. Recalibration is recommended at anytime if one of the following events occurs:-
- The lot number of reagent changes.
- Preventative maintenance is performed or a critical component is replaced.
- Control values have shifted or are out of range and a new vial of control does not rectify the problem.

QUALITY CONTROL
To ensure adequate quality control, normal and abnormal control with assayed values should be run as unknown samples:-
- At least once per day or as established by the laboratory.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.
- With every calibration.

Control results falling above the upper limit or below the lower limit of the established range indicates the assay may be out of control. The following corrective actions are recommended in such situations:-
- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results are still out of control, recalibrate with fresh calibrator, then repeat the test.
- If results are still out of control perform a calibration with fresh reagent, then repeat the test.
- If results are still out of control, contact Technical Services or your local distributor.

LIMITATIONS
1. Analytical specificity studies to determine the level of interference from various compounds that may be present in the sample were carried out on an automated clinical chemistry analyzer. The maximum interferent concentration that meets the acceptable limits of the control value (pass criterion, initial control value ± 10%), otherwise the highest interferent level tested, is reported below:
   Haemoglobin: No interference up to 1000 mg/L.
   Free Bilirubin: No interference up to 60 mg/dL (1030 µmol/L).
   Conjugated Bilirubin: No interference up to 33 mg/dL (560 µmol/L).
   Lipaemia: No interference up to 1490 mg/dL (17 mmol/L).
   Ascorbic Acid: No interference up to 82 mg/dL (4.5 mmol/L).
   Creatine: No interference up to 10 mg/dL (764 µmol/L).
   β-**Hydroxybutyrate:** No interference up to 126 mg/dL (10 mmol/L).
   Cephalothin: No interference up to 100 mg/dL (2.4 mmol/L).
   Cefotaxime: No interference up to 100 mg/dL (2.0 mmol/L).
   Acetoacetate: No interference up to 108 mg/dL (10 mmol/L).
   Proline: No interference up to 70 mg/dL (5.9 mmol/L).

EXPECTED VALUES

	Serum/Plasma[5]	**Urine**[6,7]
Adult Males:	0.62-1.10 mg/dL (55-96 µmol/L)	*40 - 278 mg/dL (3540 - 24600µmol/L) 1.0 - 2.0 g/day (8.8 - 17.7 mmol/day)
Adult Females:	0.45-0.75 mg/dL (40-66 µmol/L)	*29 - 226 mg/dL (2550-20000 µmol/L) 0.8-1.8 g/day (7.1-15.9 mmol/day)

*Values for first morning urine only.
The quoted values should serve as a guide only. It is recommended that each laboratory verify this range or derives a reference interval for the population that it serves.[8]

PERFORMANCE DATA
The following data was obtained using the Enzymatic Creatinine Reagent and unless otherwise stated, on a Roche Hitachi 911® clinical chemistry analyzer. Users should establish product performance on their specific analyzer used.

IMPRECISION
Imprecision was evaluated using the NCCLS (CLSI) EP5-A2 protocol as a guideline.[9] Studies were carried out at the same site over a period of 20 days (40 runs) using 3 levels of serum creatinine controls and 2 levels of urine creatinine controls on a single Roche Hitachi 911® clinical chemistry analyzer. Two runs per day were carried out by the same operator using a single lot of reagent and calibrator per specimen type with calibrations carried out daily.

Serum Precision		Level 1		Level 2		Level 3	
		mg/dL	µmol/L	mg/dL	µmol/L	mg/dL	µmol/L
No of Data Points (n=)		80		80		80	
Mean		0.9	83	1.6	146	5.3	468
Within Run	SD	0.02	2.0	0.03	2.8	0.04	3.3
	CV %	2.5		1.9		0.7	
Total	SD	0.03	3.0	0.04	3.3	0.07	6.1
	CV%	3.7		2.2		1.3	

Urine Precision		Level 1		Level 2	
		mg/dL	µmol/L	mg/dL	µmol/L
No of Data Points (n=)		80		80	
Mean		67.7	5980	145.3	12846
Within Run	SD	0.76	67.5	0.94	83.2
	CV %	1.1		0.6	
Total	SD	1.74	154.0	3.93	347.5
	CV%	2.6		2.7	

METHOD COMPARISON
Comparison studies were carried out using the NCCLS (CLSI) EP9-A2 protocol as a guideline.[10] Similar commercially available Creatinine reagents as recommended for use by the suppliers for their instrument systems as noted below, were used as a reference. Serum/plasma and urine samples were assayed in parallel by both the test (Y) and reference (X) methods and the results compared by Deming regression. The following statistics were obtained:

Serum/ Plasma	Hitachi 911®		SYNCRON CX9®		SYNCRON LX20®	
	mg/dL	µmol/L	mg/dL	µmol/L	mg/dL	µmol/L
n	107		81		68	
Range	0.5 - 28.5	43 - 2518	0.6 - 22.0	53 - 1945	0.5 - 22.6	44 - 1998
X mean	6.3	554	4.3	380	4.7	415
Y mean	5.9	522	4.1	359	4.5	398
Slope	0.951		0.979		0.935	
Intercept	-0.1	-5	-0.1	-13	0.1	8
r	0.9999		0.9991		0.9994	
r^2	0.9998		0.9982		0.9988	

Urine	Hitachi 911®		SYNCRON CX9®	
	mg/dL	µmol/L	mg/dL	µmol/L
n	107		104	
Range	9.8 - 136.2	865 - 12043	11.3 - 144.4	999 - 12766
X mean	63.2	5591	69.7	6160
Y mean	62.3	5507	63.2	5585
Slope	0.992		0.906	
Intercept	-0.4	-38	0.1	7
r	0.9989		0.9989	
r^2	0.9978		0.9978	

MEASURING RANGE
When run as recommended the measuring range of the assay is as follows:
Serum/Plasma: 0.2 - 30 mg/dL (18 - 2650 µmol/L)
Urine: 2.1 - 150 mg/dL (189 - 13260 µmol/L)

LIMIT OF QUANTITATION
In studies carried out over two runs on two separate days, the lowest concentration of creatinine measured in a given sample matrix which did not exceed a CV of 20% (n=10 replicates) is provided below:
Serum/Plasma: 0.2 mg/dL (18 µmol/L)
Urine: 2.1 mg/dL (189 µmol/L)

ANALYTICAL SENSITIVITY
When run as recommended, the sensitivity of this assay is 21.4 ΔmAbs per mg/dL or 0.242 ΔmAbs per µmol/L(1cm light path, 546 nm).

REFERENCES

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Fisher Diagnostics
a division of Fisher Scientific Company, LLC
a part of Thermo Fisher Scientific Inc.
Middletown, VA 22645-1905 USA
Phone: 800-528-0494
540-869-3200
Fax: 540-869-8132

EC REP MDCI Ltd.
Arundel House
1 Liverpool Gardens
Worthing, West Sussex BN11 1SL UK

CE

REF **Reorder Information**

Catalogue No.	REAG 1	REAG 2
TR35401	2 x 30 mL	2 x 10 mL