Enzymatic Creatinine Reagent Kit
MD-101112

Intended Use
For the in vitro quantitative measurement of creatinine in urine.
NOT FOR USE IN UNPROFESSIONAL SETTINGS.

Summary and Principle
Urinary creatinine is used to estimate creatinine clearance, which helps calculate the glomerular filtration rate (GFR), a key indicator of how well the kidneys are filtering waste. Low urinary creatinine can indicate kidney damage or impaired filtration.
Normalizing Other Urine Tests: many urine test results (like protein, albumin, or drug levels) are expressed as a ratio to creatinine (e.g., albumin-to-creatinine ratio, ACR).
This enzymatic method for creatinine utilizes a multi-step approach ending with a photometric end-point reaction. The enzyme creatinine amidohydrolase is used to convert
creatinine to creatine. Creatine is broken down to sarcosine and urea by creatine amidinohydrolase. Further enzyme linked steps with sarcosine oxidase and peroxidase yield a colored chromogen read at 545nm.[1] Creatinine measurements are used in the diagnosis and treatment of kidney diseases, in the monitoring of dialysis patients, and as an integral part of diagnostic equations to measure renal function.

Reagents
Both R1 and R2 reagents are provided in ready-to-use format. No preparation is required.
R1 reagent contains:
>12.000 U/L Creatine Amidinohydrolase (microbial), >4.000 L Sarcosine Oxidase (microbial), >0.24 mmol/L N-ethyl-N-sulfopropryl-m-toluidine, Ascorbate Oxidase (botanical), Buffer (pH 7.5), Stabilizers, surfactants, and preservatives.
R2 reagent contains:
>135,000 U/L Creatinine Amidohydrolase (microbial), >1.5 mmol/L 4-aminoantipyrine, >2,000 U/L Peroxidase (botanical), 7.7 mmol/L Sodium azide, Buffer (pH 7.5), stabilizers and surfactants.

Reagent Stability and Storage
After opening, both R1 and R2 reagents are stable at 2-8°C for 8 weeks if capped when not in active use. Store reagents at 2-8°C. When stored as directed, the reagents are stable until the expiration date stated on the label. For longer periods of time, store at -20°C.

Cautions
Reagents are For Laboratory Use Only. May be harmful if inhaled or swallowed. Do not pipette by mouth.
Avoid contact with skin and eyes. In case of contact, flush area with water. Seek immediate medical attention for eyes.

Instrumentation
Mission Enzymatic Creatinine Reagent Kit are for use on Mission Diagnostics Affirm C200* and Beckman AU680 Analyzers. Refer to instrument procedure instructions in the instrument manual provided with the analyzer.

Specimen Collection and Handling
Urine is to be collected without additives. For manual dilution, dilute urine with deionized water and multiply the result by the dilution factor.

Handle all urine samples as if potentially infectious.

Quality Control
Store and handle reagents properly before and during use. Every laboratory should establish its own test requirements using controls and calibrators. Mission Diagnostics provides Calibrators to meet your program needs and which conform to NLCP Guidelines[2]:

MD-101212.01 – Enzymatic Creatinine Calibrator 1
MD-101212.02 – Enzymatic Creatinine Calibrator 2
MD-101212.03 – Enzymatic Creatinine Calibrator 3

Specificity, Limitations and Interferences
Mission Enzymatic Creatinine Reagent Kit is for the detection of creatinine in urine. False positives may be resulted from improperly stored samples and bacterial growth.

Typical Performance Characteristics
The following performance data was obtained using the AFFIRM Automated Analyzer. Other instruments may yield different performance data.

* Also known as Zybio EXC200 Analyzer

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Manufactured by: Diamond Diagnostics Inc., 333 Fiske St., Holliston, MA 01746
Technical Assistance Call: 1.508.429.0450
Email: support@diamonddiagnostics.com

Linearity
The following results were obtained on an AFFIRM Automated Analyzer using the Mission Enzymatic Creatinine Reagent Kit on samples containing 2, 25, 100, 200, 300, 400 and 500 mg/dL creatinine. The table below includes mean, standard deviation (SD) and Coefficient of Variation (CV) for each value.

Mean (mg/dL)	SD	CV%
1.914	0.030	1.6
25.668	0.153	0.6
103.702	0.894	0.9
203.260	2.522	1.2
292.172	3.210	1.1
376.570	3.025	0.8
447.468	5.698	1.3

Precision
Studies performed on an AFFIRM Automated Analyzer. The precision of the assay was evaluated following a modification of NCCLS protocol EPT-T2. The within-run precision data was obtained by running two samples in replicates of 20 on the same day. The run-to-run data was obtained by running two samples in replicates over a five-day period.

Within-Run			Run-to-Run		
Mean (mg/dL)	**SD**	**CV%**	**Mean** (mg/dL)	**SD**	**CV%**
59.139	0.827	1.4	67.750	0.918	1.4
141.345	5.150	3.6	146.865	5.676	3.9

Analytical Specificity
Cross contamination studies have not been performed on AFFIRM Automated Instruments. Certain reagent/instrument combinations used in sequence with this assay may interfere with reagent performance and test results. The existence of, or effects of, any potential cross contamination issues are unknown.

Test Conditions
For the data presented in this insert, studies using this reagent were performed on AFFIRM Automated Analyzer using the parameters listed below.

Limitations
A sample with a creatinine concentration exceeding the analytical measuring range should be diluted with deionized water and reanalyzed incorporating the dilution factor in the calculation of the value.

Calibration
Calibration material should be used to calibrate the procedure. The frequency of calibration using an automated system is dependent on the system and the parameters used. If control results are found to be out of range, the test may need to be re-calibrated. Under typical operating conditions manufacturer calibration stability studies have shown the calibration curve will be stable for at least 14 days.

Method Parameters

Analyzer Specific Settings

Method type:	Kinetic (3-pt)
Slope:	positive
Units	mg/dL
DOM wavelength	546
SUB wavelength	800
Sample volume	2.0
R1 volume	120
R2 volume	100
Blank rxn read (cycles)	-
Sample rxn read (cycles)	31 - 35

Calibration Settings

Calibration type	Linear
Reagent Blank required	No
Calibrator 1	2
Calibrator 2	25
Calibrator 3	125

Analytical Measuring Range (AMR)

Range: (low)	2
Range: (high)	500

BIBLIOGRAPHY
1. K.Rikitake, I.Oka, M.Ando, T.Yoshimoto, & D. Tsuru; J. Biochem., 86, 1109 (1979)
2. National Laboratory Certification Program. General Laboratory Inspection Checklist / Report. OMB No. 0930-0158. November 2002.

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