Creatinine (Enzymatic) Reagent Set

Intended Use
For the quantitative enzymatic determination of creatinine in serum and urine on the Mindray BS-480. For In Vitro Diagnostic Use Only.

Principle
Creatinine is a catabolic product of creatine, which is used in skeletal muscle contraction. The daily production of creatine, and subsequently creatinine, depends on muscle mass, which fluctuates very little. Creatinine is excreted entirely by the kidneys and therefore is directly proportional to renal excretory function. Thus with normal renal excretory function, the serum creatinine
level should remain constant and normal. Only renal disorders, such as glomerulonephritis, pyelonephritis, acute tubular necrosis, and urinary obstruction, will cause an abnormal elevation in creatinine.[1]

The current method employs a two reagent system which eliminates interference by endogenous creatine and ascorbic acid.

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

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

$$\text{Sarcosine} + H_20 + 0_2 \xrightarrow{\text{Sarcosine oxidase}} \text{Glycine} + HCHO + H_2O_2$$

$$2H_2O_2 + \text{4-aminoantipyrine} + {}^*\text{ESPMT} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine Dye} + 4\ H20$$

*ESPMT: N-ethyl-N-sulfopropryl-m-toluidine

Reagents
Creatinine Enzyme Buffer Reagent (R1): Good Buffer (pH 7.4) 25 mmol/L, Creatine amidinohydrolase > 25 KU/L, Sarcosine oxidase > 7 KU/L, Ascorbate oxidase > 4 KU/L, ESPMT 140 mg/L

Creatinine Enzyme Color Reagent (R2): Good Buffer (pH 7.3) 100 mmol/L, Creatinine amidohydrolase > 250 KU/L, Peroxidase > 5 KU/L ,
4-aminoantipyrine 600 mg/L, ESPMT

Reagent Preparation
Reagents are provided as ready to use liquids.

Reagent Storage and Stability
Reagents are stable until expiration dates found on their labels when stored at 2-8°C. Manufacturer studies have shown reagent is stable for 30 days once placed in the refrigerated reagent carousel (2-10°C), however reagent stability may vary based on individual laboratory conditions.

Precautions and Hazards
Hazards:
R1 and R2:
Hazard Classifications: Not a hazardous substance or mixture.
Pictogram: Not required.
Signal Word: Not required.
Hazard Statements: Not a hazardous substance or mixture.
Precautionary Statements: Not a hazardous substance or mixture.

Specimen Collection and Storage
1. Serum: Remove specimen from clot promptly to prevent hemolysis.
2. Do not use fluoride or ammonium heparinate to collect sample.[2]

Sample Stability: Creatinine values have a reported stability of one day at 2-8°C, and several months when frozen (-20°C) and protected from evaporation and contamination. Store urine at 2 -8°C.[2]

Interferences
No interference was observed by ascorbic acid up to 200 mg/dL, hemoglobin up to 500 mg/dL, bilirubin-conjugate up to 32 mg/dL, and bilirubin-free up to 40 mg/dL. An extensive list of drugs or other agents interfering with creatinine methodologies
has been reported by Young et al[3].

Materials Provided
1. Creatinine R1 Reagent
2. Creatinine R2 Reagent

Materials Required but not Provided
1. Mindray BS-480
2. BS-480 Operation Manual
3. Chemistry control, catalog number CHEQ480
4. Chemistry Calibrator, catalog number CHEC480

Calibration
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.

Quality Control
Two (2) levels of control material with known Creatinine levels determined by this method, should be analyzed each day of testing.

Expected Values[4]
Normal Range: Male (serum): 0.9 - 1.5 mg/dL
Male (urine): 1000 - 2000 mg/24hrs.
Female (serum): 0.7 - 1.4 mg/dL
Female (urine): 600 - 1500 mg/24hrs.

This range should serve only as a guideline. It is recommended that each laboratory establish its own range of expected values, since differences exist between instruments, laboratories and local populations.

Performance[5]
1. Assay Range: 0.01-30.00 mg/dL. Samples exceeding this value should be diluted 2-fold with deionized water, the assay repeated and results multiplied by 2.
2. Correlation: A study was performed between the Mindray BS-480 and a similar analyzer using this method, resulting in the following:

Method	Creatinine
N	80
Mean Creatinine (mg/dL)	3.945
Range (mg/dL)	0.49-22.73
Standard Deviation	5.725
Regression Analysis	y = 1.046x + 0.036
Correlation Coefficient	0.9994

3. Precision: Precision studies were performed following a modification of the guidelines contained in the NCCLS document EP5-T2.

Within Day

Sample	LOW	MID	HIGH
N	20	20	20
Mean	1.262	4.141	24.984
Standard Deviation	0.011	0.019	0.064
Coefficient of Variation (%)	0.9%	0.5%	0.3%

Total

Sample	LOW	MID	HIGH
N	40	40	40
Mean	1.286	4.212	25.161
Standard Deviation	0.021	0.055	0.359
Coefficient of Variation (%)	1.6%	1.3%	1.4%

4. Sensitivity**:** 2SD limit of detection (95% Conf) = 0.01 mg/dL

   Data obtained on Hitachi 717
5. Urine specimens (n = 37) were assayed by this method and by another commercial method. Statistical analysis revealed a correlation coefficient (r) of 0.9854, with a regression equation of y = 1.0545x + 0.3607.

References
1. Pagana, KD and TJ Pagana, Mosby's Diagnostic and Laboratory Test Reference, 2nd Ed., Mosby, St. Louis, 1995, p.270.
2. Tietz, Norbert W, Clinical Guide to Laboratory Tests, 3rd Edition, WB Saunders, Philadelphia, 1995, pp 186-188.
3. Young DS et al. Clin Chem 21:286 D, 1975 (Special Issue)
4. Larsen K. Clin Chim Acta 41:209, 1972
5. Manufacturer's Laboratory Data

MEDTEST DX
A MedTest Company

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CHEMISTRY PARAMETERS

Chem:	CRET-enz		No.: 213	Sample Type:	Serum		
Chemistry:	Creatinine (Enzymatic)			Print Name:	CRET-enz		
Reaction Type:	End Point			Reaction Direction:	Positive		
Pri Wave:	546			Sec Wave:	660		
Unit:	mg/dL			Decimal	0.01		
Blank Time:	47	49		Reaction Time:	80	82	

	Sample Vol.	Aspirated	Diluent		Reagent Vol.	Diluent
Standard:	2.0 ul	--- ul	--- ul	R1:	120 ul	--- ul
Decreased:	--- ul	--- ul	--- ul	R2:	40 ul	-- ul
Increased:	--- ul	--- ul	--- ul	R3:	--- ul	-- ul
	☐ Sample Blank	☑ Auto Rerun		R4:	--- ul	--- ul

Slope/Offset Adjustment

Slope: 1 Offset: 0

Linearity Range (Standard)	0.01	30	Linearity Limit:
Linearity Range (Decreased)	____	____	Substrate Depletion:
Linearity Range (Increased)	____	____	Mixed Blank Abs:
R1 Blank Abs:	____	____	Uncapping Time
Blank Response:	____	____	Reagent Alarm Limit:
Twin Chemistry:			☐ Enzyme Linear Extension

☐ Prozone Check ○ Rate Check ○ Antigen Addition

Q1: Q2: Q3: Q4:

PC: ABS:

CALIBRATION PARAMETERS

Calibrator Definition

Calibrator: * Lot No.: *

Exp Date: *

Carousel	**Pos**
Sample Carousel 1	*
Sample Carousel 2	
Sample Carousel 3	

Reagent/Calibration

Calibrator	Pos	Lot No	Exp Date	Chem	Conc	Unit
Water	W	*	*	CRET-enz	0	mg/dL
Chemistry Calibrator	*	*	*	CRET-enz	*	mg/dL

Calibration Setup

Chem: CRET-enz

Calibration Settings

Math Model: Two-Point Linear

Factor: Replicates: 2

Acceptance Limits

Cal Time:	*	Hour	
Slope Diff:	---	SD:	---
Sensitivity :	---	Repeatability:	---
Deter Coeff:	---		

Auto Calib.

☐ Bottle Changed ☐ Lot Changed ☐ Cal Time

It is recommended that two levels of control material be assayed daily.
* Indicates user defined parameter.

REF CEE480

Manufactured for MedTest DX
5449 Research Drive Canton, MI 48188

2°C – 8°C IVD

Symbol Key

Use by (YYYY-MM-DD)	Temperature limitation
LOT Lot and batch code	Consult instructions for use
REF Catalog number	IVD *In vitro* diagnostic medical device
Manufacturer	