HORIBA Instruments Incorporated
5449 Research Drive, Canton, MI 48188
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Pointe Creatine Kinase Reagent Set

Intended Use
For the quantitative determination of creatine kinase activity in serum, using the Yumizen C560 analyzer. For *in vitro* diagnostic use only. **Rx Only.**

Summary and Principle
Serum creatinine kinase (CK) levels have proven valuable in the assessment of cardiac and skeletal muscle diseases, including myocardial infarction and muscular dystrophy.[1] Determination of creatine kinase and lactate dehydrogenase isoenzymes provides a definitive diagnosis of acute myocardial infarction.[2]

The kinetic procedure presented is a modification of Szasz[3] of the Rosalki[4] technique, which optimizes the reaction by reactivation of CK activity with N-actyl-L-cysteine (NAC).

CK specifically catalyzes the transphosphorylation of ADP to ATP. Through a series of coupled enzymatic reactions, NADPH is produced at a rate directly proportional to the CK activity. The method determines the NADPH absorbance increase per min at 340 nm.

Reagents
CK R1 (buffer) contains: Imidazole buffer (pH 6.7) 100.0 mmol/L; NADP 2.0 mmol/L; HK (Baker's yeast) 2.5 KU/L; Glucose 20.0 mmol/L; Magnesium Acetate 10.0 mmol/L; EDTA 2.0 mmol/L and N-acetylcysteine (NAC) 20.0 mmol/L.

CK R2 (enzyme reagent) contains: Imidazole buffer (pH 6.7) 100.0 mmol/L; ADP 2.0 mmol/L; AMP 5.0 mmol/L; Diadensosine pentaphosphate 10.0 mmol/L; Creatine phosphate 30.0 mmol/L; G_6PDH (Baker's yeast) 1.5 KU/L and EDTA 2.0 mmol/L.

Reagent Preparation
Reagents are supplied as ready to use liquids.

Reagent Storage
1. Reagents should appear clear and colorless. Discard if either appears cloudy or contains particulate matter.
2. Store R1 and R2 at 2-8°C, protected from light. If stored as directed the reagents are stable until the expiration date.
3. 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
1. This reagent is for *in vitro* diagnostic use only.
2. Normal precautions in handling laboratory reagents should be followed.
3. The reagents contain sodium azide which may be toxic if ingested. Sodium azide may also react with lead and copper plumbing to form highly explosive metal azides. Refer to Material Safety Data Sheet for any updated risk, hazard or safety information.

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. **Refer to the Safety Data Sheet for this product (SDS-C7522) available by calling 1-734-487-8300.**

Specimen Collection and Handling
1. Clear unhemolyzed serum is the specimen of choice. No special additives or preservatives are required.
2. Whenever possible, specimens should be separated and analyzed on the day of collections and stored in capped tubes.
3. CK activity in serum is reportedly stable for three days at 2-8°C. Addition of sulfhydryl agents preserves CK activity during prolonged storage.[5,6] Some control sera, however, show a considerable decrease in CK activity only a few hours after reconstitution.

Interferences
1. Intramuscular injections and strenuous physical exercise may elevate serum CK.
2. Chloride and sulfate inhibit CK activity.
3. Bilirubin levels up to 40 mg/dl and triglyceride levels up to 1550 mg/dl show no interference in this test.[9]
4. Young, et al. have reviewed drug effects on serum CK levels.[7]

Materials Provided
CK R1 and R2 Reagent.

Materials Required but not Provided
1. Yumizen C560
2. Yumizen C560 Operation Manual
3. Chemistry control, catalog number C7592-100

Calibration
CK activity is based on the "micromolar extinction coefficient" of NADP at 340 nm. The instrument manufacturer's calibration guidelines should be followed to calibrate your analyzer. Assaying the CK contents of a control serum with known CK values can be used to assure instrument calibration has been performed correctly.

Limitations

If the ΔAbs./min is greater than 0.345, dilute 1 part sample with 9 parts saline and re-assay. Multiply results by 10. CK values for neonatal patients have not been established with this procedure.

Quality Control

The validity of the reaction should be monitored by use of control sera with known normal and abnormal creatine kinase values. These conditions should be run at least with every working shift in which creatine kinase assays are performed. It is recommended that each laboratory establish its own frequency of control determination.

Expected Values[8]

Normal range: Males: 38-174 U/L (37°C)
Females: 26-140 U/L (37°C)

The 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 Characteristics[9]

1. Assay Range: 1-1200 U/L Performed according to NCCLS Guideline EP6-P.
2. Comparison: A study was performed between the Yumizen C560 and a similar analyzer using this method, resulting in the following:

Method	Creatine Kinase
N	87
Mean CK (U/L)	185.2
Range (U/L)	5-1019
Standard Deviation	243.3
Regression Analysis	y = 0.994x – 5.9
Correlation Coefficient	0.9946

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	135.6	275.8	953.0
Standard Deviation	1.5	1.4	2.5
Coefficient of Variation (%)	1.1%	0.5%	0.3%

Total

Sample	LOW	MID	HIGH
N	40	40	40
Mean	114.1	251.3	958.2
Standard Deviation	3.6	4.2	7.0
Coefficient of Variation (%)	3.2%	1.7%	0.7%

4. Sensitivity: 2SD limit of detection (95% Conf) = 1 U/L

References

1. Kachmar JF,, Moss DW., In Fundamentals of Clinical Chemistry, 2nd ed. NW Tietz, Editor. WB Saunders, Philadelphia, 1976, p 682.
2. Row CR et al., J Lab Clin. Med., 80:557, 1972.
3. Szasz G., Proceedings of the Second International Symposium on Clinical Enzymology, Chicago, October 1975.
4. Rosalki S.B., J Lab Clin. Chem., 23:646, 1977.
5. Morin LG, Clin. Chem., 23:646, 1977.
6. Nealon DA, Henderson AR., Clin. Chem., 23:646, 1977.
7. Young DS et al., Clin. Chem., 21: 286D, 1975 (Special Issue).
8. Tietz, Norbert W., Clinical Guide To Laboratory Tests, W.B. Saunders Company, Philadelphia, PA., (1995), p180.
9. Manufacturer's Laboratory Data

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CHEMISTRY PARAMETERS

Chem:	CK	No.:	211	Sample Type:	Serum
Chemistry:	Creatine Kinase			Print Name:	CK
Reaction Type:	Kinetic			Reaction Direction:	Positive
Pri Wave:	340			Sec Wave:	412
Unit:	U/L			Decimal	0
Blank Time:	0	0		Reaction Time:	56 71

	Sample Vol.	Aspirated	Diluent	Reagent Vol.	Diluent
Standard:	3.3 ul	--- ul	--- ul	R1: 120 ul	--- ul
Decreased:	3.3 ul	20 ul	180 ul	R2: 30 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)	1	1200	Linearity Limit:	0.3
Linearity Range (Decreased)	10	12000	Substrate Depletion:	25000
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:

Pointe
Creatine Kinase
Reagent Set

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	*	*	CK	0	U/L

Calibration Setup

Chem:	CK		
Calibration Settings			
Math Model:	K Factor		
Factor:	6158	Replicates:	1
Acceptance Limits			
Cal Time:	24	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 14-C7522-220

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2°C 8°C IVD

Certified to Perform Reagents

The Pointe reagents are certified to be manufactured according to specified parameters. Any Pointe reagent product not meeting specifications through its listed expiration date will be remedied immediately without charge.

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CE

Symbol Key

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