CREATINE KINASE

(KINETIC)

4 + 1

INTENDED USE

Vitro CPK reagent is intended for the in vitro quantitative determination of Creatine Kinase (CK) in human serum and plasma.

METHOD

Kinetic UV method according to IFCC specifications.

Liquid stable reagent.

BACKGROUND

Creatine kinase (CK) is a dimeric enzyme occurring in four different forms: a mitochondrial isoenzyme and the cytosolic isoenzymes CK-MM (muscle type), CK-BB (brain type) and CK-MB (myocardial type). The determination of CK and CK-isoenzyme activities is utilized in the diagnosis and monitoring of myocardial infarction and myopathies such as the progressive Duchenne muscular dystrophy. Following injury to the myocardium, such as occurs with acute myocardial infarction. CK is released from the damaged myocardial cells. In early cases, a rise in the CK activity can be found just 4 hours after an infarction,, the CK-activities reaches a maximum after 12-24 hours and then falls back to the normal range after 3-4 days. Myocardial damage is very likely when the total CK activity is above 190 U/l, the CK-MB activity is above 24 U/l (+37°C) and the CK-MB activity fraction exceeds 6% of the total.

ASSAY PRINCIPLE

The assay method using creatine phosphate and ADP was first described by Oliver, modified by Rosalki and further improved for optimal test conditions by Szasz. CK is rapidly inactivated by oxidation of the sulfhydryl groups in the active center. The enzyme can be reactivated by the addition of acetylcysteine (NAc). Interference by adenylate kinase is prevented by the addition of diadenosine pentaphosphate and AMP. Standardized methods for the determination for CK using the "reverse reaction" and activation by NAc were recommended by the German Society for Clinical Chemistry (DGKC) and the International Federation of Clinical Chemistry (IFCC) in 1977 and 1990 respectively. This assay meets the recommendations of the IFCC and DGKC (Standard method 94).

The series of reactions involved in the assay system is as follows:

Creatine phosphate + ADP $\xleftrightarrow{CK\ (AMP,\ NAC)}$ **Creatine + ATP**

G6PD +NADP+H_2O $\xleftrightarrow{G6P\text{-}DH}$ **Gluconate-6P + NADPH?+H^+**

The rate of reduction of the coenzyme NADP is proportional to the CPK activity in the specimen. It is determined by measuring the increase in absorbance at 334 / 340 / 365 nm correspondingly.

EXPECTED VALUES

	Male	Female
25°C	Up to 65 U/l Up to 1083 µkat/l	Up to 55 U/l Up to 917 nkat/l
30°C	Up to 105 U/l Up to 1750 µkat/l	Up to 80 U/l Up to 2500 nkat/l
37°C	Up to 174 U/l Up to 2900 µkat/l	Up to 225 U/l Up to 3750 nkat/l

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes, the ALT results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

REAGENTS

R_1	Imidazol buffer pH 6.7	100 mmol/l
	Glucose	20 mmol/l
	NAC	20 mmol/l
	Magnesium acetate	10 mmol/l
	NADP	2.5 mmol/l
	Hexokinase	4 KU/l
	EDTA	2 mmol/l
R_2	Creatine phosphate	30 mmol/l
	ADP	2 mmol/l
	Diadenosine-5'pentaphosphate	10 µmol/l
	G6PDH	1.5 KU/l

- **Reagent Preparation & Stability**

All reagents are ready for use and stable up to the expiry date given on label when stored at 2–8°C.

Mix 4 ml of R_1 + 1 ml of R_2

Mix well, do not shake. the working solution is stable for:

10 days at 20–25 °C.

4 weeks at 2? – 8 °C

SPECIMEN

Serum, is the only acceptable material. EDTA or heparinized plasma. Avoid hemolysis can produce erroneous results.

- **Specimen Preparation & Stability**

Separate serum from clot/cells immediately. CPK is stable for 8 days at 2 – 8°C or one month if stored at -20°C

PROCEDURE

- **Manual Procedure**

Wavelength 340 nm

Cuvette 1 cm light path

Temperature 25, 30 or 37 °C

Zero adjustment against air

Pipette into test tube or cuvette			
	25°C	30°C	37°C
Working solution	1 ml	1 ml	1 ml
Serum	40 µl	40 µl	20 µl

Mix, incubate for 1.0 minute, and start stopwatch simultaneously. Read again after exactly 1, 2, and 3 minutes.

- **Automated Procedure**

User defined parameters for different auto analyzers are available upon request.

CALCULATION

Determine the change in absorbance per minute (ΔA/min) from the linear portion of the reaction curve and calculate the CPK activity by using the following formulae:

U/l (25°C/30°) 4127 × ΔA 340 nm/min

U/l (37°) 8095 × ΔA 340 nm/min

One international unit **(U)** is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under specified conditions.

The general formula for converting ΔA/min into U/l is:

$$U/l = \frac{\Delta A/min \times TV \times 1000}{*\sum \times SV \times LP}$$

Where:

TV	Total reaction volume in ml
SV	Sample volume in ml
*∑	millimolar absorptivity of NADH
LP	Cuvette pathlength in cm
1000	Conversion of U/ml to U/l

* millimolar absorptivity of NADH

at 334 nm= 6.18,

at 340 nm= 6.22, and

at 365 nm= 6.40

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- **Unit conversion**

U/l x 16.67 x 10^{-3} = µl

QUALITY CONTROL

It is recommended that controls (normal and abnormal) be included in:

- Each set of assays, or
- At least once a shift, or
- When a new bottle of reagent is used, or
- After preventive maintenance is performed or a clinical component is replaced.

Commercially available control material with established CPK values may be routinely used for quality control.

Failure to obtain the proper range of values in the assay of control material may indicate:

- Reagent deterioration,
- Instrument malfunction, or
- Procedure errors.

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 on fresh control material still remain outside the limits, then repeat the test with fresh reagent.

- If results are still out of control, contact Vitro Technical Services.

INTERFERING SUBSTANCES

- **Anticoagulants:**
  Fluoride and citrate inhibit the enzyme activity. The only accepted anticoagulants are heparin and EDTA.
- **Bilirubin:**
  No interference from free bilirubin up to a level of 15 mg/dl, and from conjugated bilirubin up to level of 6.8 mg/dl.
- **Drugs:**
  Young[7] in 1990 has published a comprehensive list of drugs and substances which may interfere with this assay.
- **Haemolysis:**
  Erythrocyte contamination may elevates results, since CPK activities in erythrocytes are three to five times higher than those in normal sera.
- **Lipemia:**
  Lipemic specimens may cause high absorbance flagging. Choose diluted sample treatment for automatic rerun.

WARNING & PRECAUTION

- Vitro CPK reagent is for in vitro diagnostic use only. Normal precautions exercised in handling laboratory reagents should be followed.
- Warm up working solution to the corresponding temperature before use.
- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- Valid results depend on an accurately calibrated instrument, timing, and temperature control.

PERFORMANCE CHARACTERISTICS

Imprecision

Reproducibility was determined using in an internal protocol. The following results were obtained.

	Within Run		Between Day	
Control	Level I	Level II	Level I	Level II
Number of samples	40	40	40	40
Mean (U/l)	134	455	133	432
SD (U/l)	2.4	4.0	3.6	15.6
CV (%)	1.78	0.92	2.2	4.1

Method Comparison

Comparison studies were carried out using another similar commercially available CK-NAC reagent as a reference. Serum samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained:

Number of sample pairs 45
Range of sample results 25 - 561 U/l
Mean of reference method results 141 U/l
Mean of CK-NAC results 140 U/l
Slope 0.997
Intercept 2.5 U/l
Correlation coefficient 0.9995

Sensitivity

The sensitivity is defined as the lower detection limit represents the lowest measurable CPK activity that can be distinguished from zero.

When run as recommended the sensitivity of this assay is 4 U/l.

LINEARITY

When run as recommended, the assay is linear up to 2100 U/l.

If result exceeds 2100 U/l, specimen should be diluted 1+5 with 0.9% NaCl solution and reassayed. Multiply the result by 6.

BIBLIOGRAPHY

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SYMBOL DECLARATION

	Manufacturer
	Consult instructions for use
LOT	Batch code (Lot #)
REF	Catalog number
	Temperature limitation
IVD	In vitro diagnostic medical device
	Use by
	Caution. Consult instructions
	Keep away from light

ORDERING INFORMATION

REF	SIZE
1131	5 X 10 ml
1132	10 X 10 ml
1133	4 X 20 ml
1134	3 X 50 ml
1135	10 X 20 ml

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