CREATINE PHOSPHOKINASE (CK-NAC)

Determination of creatine kinase in serum and plasma based on recommendation of DGKC

TEST SUMMARY

The enzyme examined catalyses the hydrolysis of Creatine Phosphate forming ATP, which transforms Glucose to Glucose-6-phosphate.
Glucose-6-phosphate reacts with NADP converting it to NADPH causing an increasing of absorbance at 340 nm.

SAMPLES

Serum is the preferred specimen. Plasma containing Heparin, EDTA, citrate, or fluoride may produce unpredictable reaction rates. CK activity in serum is unstable and is rapidly lost during storage. CK is inactivated both by bright daylight and by increasing specimen pH owing to loss of carbon dioxide; accordingly, specimens should be stored in the dark in tightly closed tubes. CK is susceptible to thermal denaturation; the degree of inactivation corresponds to the degree of temperature increase. Therefore, the serumspecimen should be chilled to 4°C as rapidly as possible after collection. A slight degree of hemolysis can be tolerated because erythrocytes contain no CK activity. However, moderately or severely hemolyzed specimens are unsatisfactory because anzymes and intermediates liberated from the erythrocytes may affect the lag phase and the side reactions occurring in the assay system.

REAGENTS

Reagent A: Imidazole buffer 0.1 M pH 6.7; Magnesium Acetate 10 mM; Glucose 20 mM; preservatives and stabilizers.

Reagent B: ADP 2 mM; AMP 10 mM; adenosinepentaphosphate 0.01 mM; NADP 2 mM; Exokinase (HK) ≥ 2000 U/l;Glucosio-6-fosfato Dehydrogenase (G6P-DH) ≥ 1000 U/l; N-Ac N-Acetyl-L-cysteine (NAC) 20 mM; EDTA 2 mM; Creatinephosphate 20 mM; preservatives and stabilizers.

MATERIALS REQUIRED BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micropipettes. Glass or high quality polystyrene cuvettes. Physiological solution.

PRECAUTIONS

Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.
Perform the test according to the general "Good Laboratory Practice" (GPL) guidelines.

REAGENTS PREPARATION

PROCEDURE STARTER SAMPLE
Add 6 ml of Reagent B to a vial of Reagent A. Work's reagent is stable 30 days at 2-8°C away from light sources

PROCEDURE STARTER REAGENT
Use reagents separately.
Stability: until expiration date on label away from light source.
Stability after first opening: ≥ 60 days.

PROCEDURE (STARTER SAMPLE)

Kind of analysis:	Kinetics (increasing)
Reading time:	1,2,3, minutes
Delay:	60 sec.
Wavelength:	340 nm
Temperature:	37°C
Lenghtpath:	1 cm
Zero:	Distilled water

REAGENTS	CUVETTE
Work reagent	1 ml
Preincubate at 37 °C at least for 5 minutes	
Sample	40 µl

PROCEDURE (STARTER REAGENT)

Kind of analysis:	Kinetics (increasing)
Reading time:	1,2,3, minutes
Delay:	60 sec.
Wavelength:	340 nm
Temperature:	37°C
Lenghtpath:	1 cm
Zero:	Distilled water

REAGENTS	CUVETTE
Reagent A Sample	1 ml 50 µl
Preincubate at 37 °C at least for 5 minutes	
Reagent B	250 µl

CALCULATION

Activity in U/l: ΔA/min x 4127

Activity in µkat/l: U/l x 0.0167

EXPECTED VALUES

Men	24 - 204 U/l	(0.39 - 3.40 µkat/l)
Women	24 - 173 U/l	(0.39 - 2.90 µkat/l)

Each laboratory should establish appropriate reference intervals related to its population.

NOTE

- If the results are incompatible with clinical presentation, they have to be evaluated within a total clinical study.
- Only for IVD use.

CALIBRATION/QUALITY CONTROL

It is suggested to perform an internal quality control with control sera are available.

TEST PERFORMANCE

Precision

Intra-assay (n = 10)	Mean (U/l)	SD (U/l)	CV%
Sample 1	148.34	1.33	0.90
Sample 2	461.34	4.62	1.00

Inter-assay (n = 20)	Mean (U/l)	SD (U/l)	CV%
Sample 1	148.21	0.94	0.64
Sample 2	464.75	3.98	0.86

Sensibility/limit of detection
Method is able to discriminate up to 1 U/l.

Linearity
Method is linear up to 2000 U/l.
If ΔA/min is exceeded by 0.250, is suggested to dilute sample 1+9 with saline and to repeat the test, multiplying the results by10.

Methods comparison
A comparison with a commercial available product gave the following results in a comparison on 100 samples:

CK-NAC LTA = x
CK-NAC competitor = y
n = 100

y = 1,04x - 3,10 U/l r = 0,9985

Interferences
No interference was observed by the presence of:

hemoglobin	≤ 400 mg/dl
bilirubin	≤ 40 mg/dl

Lipids interferences are possible for CK values in normality interval. Samples of CK values which exceed that limit have not showed lipids interferences for values ≤ 1000 mg/dl.

WASTE DISPOSAL

Product is intended for professional laboratories.
Waste products must be handled as per relevant security cards and local regulations.

PACKAGING

CODE CC01300	(120 TESTS)	
Reagent A	4 x 24 ml	(liquid)
Reagent B	1 x 24 ml	(liquid)

REFERENCES

HU Bergmeyer – Methods of enzymatic analysis, Vol. III (1987).
DGKC – Eur.J.Clin.Chem.Biochem., 31 (1993).
Tietz Textbook of Clinical Chemistry, Second Edition, Burtis-Ashwood (1994).

MANUFACTURER

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SYMBOLS

IVD Only for IVD use
LOT Lot of manufacturing
REF Code number
Storage temperature interval
Expiration date (year, month)
Warning, read enclosed documents
Read the directions
Biological risk

Mod. 01.06 (ver. 4.3 – 12/05/2008)

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