CREATINE PHOSPHOKINASE (CK-NAC)

Kinetic determination of creatine kinase (CK) in serum and plasma with IFCC-DGKC optimized method

TEST SUMMARY

Creatine kinase (CK) is an enzyme found in the heart, brain and skeletal muscle.
CK catalyzes both the conversion of creatine to phosphocreatine and the reverse reaction.
When muscle damage occurs, increased amounts of CK are released into the blood within a few hours.
The concentration peaks between 12 and 24 hours and returns to normal after 2-4 days. If further damage occurs, CK concentrations can remain high.
The increase in CK values may be associated with myocardial infarction, acute cerebrovascular forms, traumas or diseases of the muscular system.

PRINCIPLE OF THE TEST

Creatine kinase present in the sample catalyzes the hydrolysis of Creatine Phosphate with formation of ATP, which transforms Glucose into Glucose-6-phosphate.
Glucose-6-phosphate is oxidized by Glucose-6-phosphate dehydrogenase, with reduction of NADP in NADPH. The formation of NADPH causes an increase in absorbance at 340 nm directly proportional to the serum activity of the CK.

SAMPLES

Serum, heparin or EDTA plasma.
Do not use hemolyzed samples.
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.
Stable 7 days at 2-8°C or 28 days at -20°C.

REAGENTS

Reagent A: Imidazole buffer 110 mmol/l, N-Acetyl-L-cysteine (NAC) 35 mmol/l, Magnesium Acetate 10 mmol/l, Glucose 20 mmol/l, HK ≥ 4 KU/l, EDTA 2 mmol/l, NADP 2.5 mmol/l, pH 6.3.

Reagent B: Imidazole buffer 110 mmol/l, Creatinephosphate 150 mmol/l, ADP 20 mmol/l, EDTA 2 mmol/l, G6P-DH ≥ 5 KU/l, pH 9.1.

MATERIALS REQUIRED BUT NOT SUPPLIED

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

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

Mix 4 parts of Reagent A with a part of Reagent B or add 6 ml of Reagent B to a vial of Reagent A.
Prepare the necessary amount of working reagent for the analytical session, keep it protected from light.

PROCEDURE STARTER REAGENT

Use reagents separately.
Use separate reagents. Stability: up to the expiration date printed on the package, stored away from direct light and not contaminated during use.

PROCEDURE (STARTER SAMPLE)

Kind of analysis:	Kinetics (increasing)
Reading time:	5, 6, 7, 8 minutes
Delay:	5 min.
Wavelength:	340 nm
Temperature:	37°C
Lenghtpath:	1 cm
Zero:	Distilled water

REAGENTS	CUVETTE
Working reagent	1 ml
Sample	40 µl
Mix and incubate at 37°C. Read the initial absorbance and repeat the readings after 1, 2, 3 minutes against Distilled Water. Calculate the ΔA / min.	

PROCEDURE (STARTER REAGENT)

Kind of analysis:	Kinetics (increasing)
Reading time:	5, 6, 7, 8, minutes
Delay:	5 min.
Wavelength:	340 nm
Temperature:	37°C
Lenghtpath:	1 cm
Zero:	Distilled water

REAGENTS	CUVETTE
Reagent B Sample	250 µl 50 µl
Preincubate at 37 °C at least for 5 minutes	
Reagent A	1000 µl
Mix and incubate at 37°C. Read the initial absorbance and repeat the readings after 1, 2, 3 minutes against Distilled Water. Calculate the ΔA / min.	

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	153	3.5	2.28
Sample 2	564	3.2	0.56
Sample 3	795	5.7	0.72

Inter-assay (n = 20)	Mean (U/l)	SD (U/l)	CV%
Sample 1	153	2.4	1.57
Sample 2	546	4.6	0.84
Sample 3	789	10.9	1.40

Sensibility/limit of detection
Method is able to discriminate up to 4 U/l.

Linearity
Method is linear up to 800 U/l.
If ΔA/min is exceeded by 0.180, is suggested to dilute sample 1+4 with saline and to repeat the test, multiplying the results by 5.

Methods comparison
A comparison with a commercial available product gave the following results:

y = 1,098x + 6,8 U/l r = 0,999

Interferences
No interference was observed by the presence of:

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

Some medicines can interfere with the CK dosage.

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

LTA s.r.l.
Via Milano 15/F
20060 Bussero (Milan) ITALY
Tel: +39 02 95409034
Fax: +39 02 95334185
e-mail: info@LTAonline.it
Website: http://www.LTAonline.it

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. 5.1 – 26/09/2019)

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