CK-NAC FL IFCC/DGKC

CK F060 CH	6 x 10 ml
CK F120 CH	12 x 10 ml
CK F245 CH	12 x 20 ml

INTENDED USE

Reagent for quantitative in vitro determination of creatine kinase in biological fluids.

SUMMARY OF TEST

Creatine kinase (CK) is an enzyme which is contained in heart, brain and skeletal muscles. Thus, an increase of circulating level of CK may be associated to myocardial infarct, acute cerebrovascular desease, trauma or diseases of skeletal muscles.

PRINCIPLE OF THE METHOD

Creatine kinase (EC 2.7.3.2; adenosine triphosphate: creatine N-phosphotransferase; CK) catalyzes the conversion of creatine phosphate and ADP to creatine and ATP. ATP and glucose are converted to ADP and glucose-6-phosphate by hexokinase. Glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate to 6-phosphogluconate, reducing NADP to NADPH. The rate of conversion of NAPD/NADPH, monitored at 340 nm, is proportional to CK activity. N-acetyl cysteine (NAC) is added as an activator of CK.

KIT COMPONENTS

For in vitro diagnostic use only.
The components of the kit are stable until expiration date on the label.
Keep away from direct light sources.

CK-NAC R1 **F060: 6 x 8 ml (liquid) blue cap**
F120: 12 x 8 ml (liquid) blue cap
F245: 12 x 16 ml (liquid) blue cap

CK-NAC R2 **F060: 1 x 12 ml (liquid) red cap**
F120: 2 x 12 ml (liquid) red cap
F245: 3 x 16 ml (liquid) red cap

Composition in the test: imidazole buffer 29 mM pH 6.50, creatine phosphate 30 mM, glucose 20 mM, N-acetyl-L-cysteine 20 mM, magnesium acetate 10 mM, EDTA 2 mM, ADP 2 mM, NADP 2 mM, AMP 5 mM, Di(adenosine-5')pentaphosphate 12 μM, glucose-6-phosphate-dehydrogenase ≥3 kU/l, hexokinase ≥3 kU/l.

Store all components at 2-8°C.

MATERIALS REQUIRED BUT NOT SUPPLIED

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

REAGENT PREPARATION

Serum as starter procedure:
Codes F060/F120: add 2 ml of reagent R2 to a vial of reagent R1.
Code F245: add 4 ml of reagent R2 to a vial of reagent R1.
Stability of working reagent: 30 days at 2-8°C, away from light sources.

Reagent as starter procedure:
use separate reagents ready to use.
Stability: up to expiration date on labels at 2-8°C;
Stability since first opening of vials: preferably within 60 days at 2-8°C.

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" (GLP) guidelines.

SPECIMEN

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 serum specimen 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 enzymes and intermediates liberated from the erythrocytes may affect the lag phase and the side reactions occurring in the assay system.

TEST PROCEDURE (sample as starter)

Wavelenght:	340 nm
Ligthpath:	1 cm
Temperature:	37°C
dispense in cuvette working reagent:	1 ml
preincubate at 37°C for 5 minutes.	
add sample:	40 μl
Mix, execute a first reading of absorbance after 1 minute, incubating at 37°C. Perform other 3 readings at 60 seconds intervals. Calculate the ΔA/min.	

TEST PROCEDURE (reagent as starter)

Wavelenght:	340 nm
Ligthpath:	1 cm
Temperature:	37°C
dispense in cuvette reagent R1:	1 ml
add sample:	50 μl
incubate at 37°C for 5 minutes.	
dispense in cuvette reagent R2:	250 μl
Mix, execute a first reading of absorbance after 1 minute, incubating at 37°C. Perform other 3 readings at 60 seconds intervals. Calculate the ΔA/min.	

RESULTS CALCULATION

Perform calculation in units per litre, multiplying the ΔA/min by the factor as it is indicated.

Calculation in U/l: ΔA/min x 4127

Activity in μkat/l: U/l x 0.0167 = μkat/l

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.

QUALITY CONTROL AND CALIBRATION

It is suggested to perform an internal quality control. For this purpose the following human based control sera are available:
QUANTINORM CHEMA
with normal or close to normal control values
QUANTIPATH CHEMA
with pathological control values.
If required, a multiparametric, human based calibrator is available:
AUTOCAL H

Please contact Customer Care for further informations.

TEST PERFORMANCE

Linearity
the method is linear up to 2000 U/l.
If a ΔA/min of 0.250 is exceeded, it is suggested to dilute sample 1+9 with saline solution and to repeat the test, multiplying the result by 10.

Sensitivity/limit of detection (LOD)
the limit of detection is 1.6 U/l.

Interferences
no interference was observed by the presence of:

hemoglobin	≤ 400 mg/dl
bilirubin	≤ 40 mg/dl
lipids	≤ 660 mg/dl

Precision

intra-assay (n=10)	mean (U/l)	SD (U/l)	CV%
sample 1	148.21	0.94	0.64
sample 2	464.75	3.98	0.86

inter-assay (n=20)	mean (U/l)	SD (U/l)	CV%
sample 1	148.35	1.33	0.90
sample 2	461.34	4.62	1.00

Methods comparison
a comparison between Chema CK-NAC FL and a commercially available product gave the following results:

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

$y = 1.04x - 3.10$ U/l $r^2 = 0.9985$

WASTE DISPOSAL

This product is made to be used in professional laboratories.
P501: Dispose of contents according to national/international regulations.

REFERENCES

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

MANUFACTURER

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SYMBOLS

IVD	*in vitro* diagnostic medical device
LOT	batch code
REF	catalogue number
	temperature limit
	use by date
	caution
	consult instructions for use

CE