

CK-NAC FL

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

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 disease, trauma or diseases of skeletal muscles.

After a myocardial infarct, CK level begins raising between 4th and 6th hour after first acute symptoms, reaching the peak between 18th and 30th hour and coming back to normal values during the 3rd day.

CK is present in three different isoenzymatic forms, which could be separated by electrophoresis or column chromatography; each form is originated in different body tissues, paying off their diagnostic determinations.

The formula of present reagent is based on DGKC and IFCC recommendations.

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 a 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.

Reagent A F120: 12 x 8 ml (liquid) blue cap
F245: 12 x 16 ml (liquid) blue cap

Reagent B 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 µM, 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:

Code F120: add 2 ml of reagent B to a vial of reagent A.

Code F245: add 4 ml of reagent B to a vial of reagent A.

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: ≥ 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)

Wavelength:	340 nm
Lightpath:	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)

Wavelength:	340 nm
Lightpath:	1 cm
Temperature:	37°C
dispense in cuvette reagent A:	1 ml
add sample:	50 µl
incubate at 37°C for 5 minutes.	
dispense in cuvette reagent B:	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:

QN 0050 CH QUANTINORM CHEMA 10 x 5 ml
with normal or close to normal control values

QP 0050 CH QUANTIPATH CHEMA 10 x 5 ml
with pathological control values.

If required, a multiparametric, human based calibrator is available:

AT 0030 CH AUTOCAL H 10 x 3 ml

Please contact Customer Care for further information.

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 U/L.

Interferences

no interference was observed by the presence of:

hemoglobin ≤ 400 mg/dl

bilirubin ≤ 40 mg/dl

Lipids interferences are possible for CK values within normal values. No interference by lipids ≤ 1000 mg/dl was observed in samples with CK values more than normal reference interval.

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	148.34	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

$$y = 1.04x - 3.10 \text{ U/l} \quad r=0.9985$$

WASTE DISPOSAL

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment. Refer to special instructions/safety data sheets.

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, Bur-tis-Ashwood (1994).

MANUFACTURER

Chema Diagnostica
Via Padre Vincenzo Pellegrini 3
60035 Jesi (AN) - ITALY - EU
phone +39 0731 213360
fax +39 0731 213361
e-mail: mail@chema.com
website: http://www.chema.com

SYMBOLS

	for in vitro diagnostic use only
	lot of manufacturing
	code number
	storage at temperature interval
	expiration date (year/month)
	warning, read enclosed documents
	read the directions

