CK-NAC UV

unitest y AA

Optimized UV method (IFCC) for the determination of Creatine Kinase (CK) in serum or plasma

SUMMARY

Creatine Kinase is an intracellular enzyme mostly found in skeletal and heart muscles, as well as in the brain. Therefore, an increase in serum activity indicates cellular damage.
In Acute Myocardial Infarction (AMI), the increase of CK serum activity starts 2 to 6 hours after the onset and reaches its peak after 18 to 24 hours. Since peaks may be 20 times the highest peak increase, this is perhaps the most sensitive test for AMI diagnosis.

PRINCIPLE

The reaction system is as follows:

$$\text{creatine phosphate} + \text{ADP} \xrightarrow{\text{CK}} \text{creatine} + \text{ATP}$$

$$\text{ATP} + \text{glucose} \xrightarrow{\text{HK}} \text{ADP} + \text{glucose-6-phosphate}$$

$$\text{glucose-6-P} + \text{NADP} \xrightarrow{\text{G-6-PDH}} \text{gluconate-6-P} + \text{NADPH} + \text{H}^+$$

In the reaction system, NAC works as activator of the Creatine Kinase, recommended by the IFCC.

PROVIDED REAGENTS

Reagent B: Imidazole buffer solution, pH 6.7.
Reagent A: vial containing dry substrate in the required quantities for the following final concentrations:

Imidazole	100 mmol/l; pH 6.7
Creatine phosphate	30 mmol/l
ADP	2 mmol/l
Glucose	20 mmol/l
NADP	2 mmol/l
Hexokinase	≥ 2500 U/l
Glucose-6-phosphate dehydrogenase	≥ 2000 U/l
Magnesium acetate	10 mmol/l
AMP	5 mmol/l
Di (adenosine-5´) pentaphosphate	10 umol/l
N-Acetyl-cysteine	20 mmol/l

INSTRUCTIONS FOR USE

Reagent B: ready to use.
Reagent A: reconstitute according to the following:
- CK-NAC UV AA: dissolve each Reagemt A vial with 20 ml Reagent B. Date.
- CK-NAC UV unitest: dissolve each Reagent A vial with 2.5 ml Reagent B. Date.

WARNINGS

Reagents are for "in vitro" diagnostic use.
Use the reagents according to the working procedures for clinical laboratories.
The reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: stable in refrigerator (2-10ºC) until the expiration date shown on the box.
Reconstituted Substrate: stable in refrigerator for 20 days (2-10ºC) or 3 days at room temperature from reconstitution date.

INSTABILITY OR DETERIORATION OF REAGENTS

When the spectrophotometer has been set to zero with distilled water, absorbance readings of the reconstituted Reagent A higher than 0.800 O.D. (at 340 nm) indicate its deterioration.

SAMPLE

Serum or plasma
a) Collection: obtain in the usual way.
b) Additives: when using plasma, use only heparin (concentration < 15 U/l) or EDTA as anticoagulant.
c) Known interfering substances: no interferences are observed from bilirubin up to 90 mg/l, triglycerides up to 13 g/l, heparin up to 50 IU/l. Sera with visible hemolysis (hemoglobin concentration > 008 g/dl) produce interferences.
See Young, D.S. in References for effect of drugs on the present method.
d) Stability and storage instructions: sample should be preferably fresh. It can be kept up to 1 week in refrigerator (2-10ºC) without preservatives.

REQUIRED MATERIAL (non-provided)

- Spectrophotometer.
- Micropipettes and pipettes for measuring the stated volumes
- Water bath at the temperature indicated under PROCEDURE.
- Stopwatch.

ASSAY CONDITIONS

(Increase of Absorbance)
- Wavelength: 340 nm (Hg 334 or 366)
- Reaction temperature: 25, 30 or 37ºC. See the REFERENCE VALUES corresponding to each temperature.
- Reaction time: varies according to the selected procedure.

PROCEDURE

Set instruments to zero with distilled water.

A) 30 - 37ºC

I- MACROTECHNIQUE

In a cuvette at 30-37ºC, place:

Reconstituted Reagent A	2.5 ml
Pre-incubate 3-4 minutes. Then add:	
Sample	50 ul

Mix immediately and wait 3 minutes. Adjust absorbance to a reference reading and simultaneously start stopwatch. Record absorbance 1, 2 and 3 minutes after first reading. Determine average change in Absorbance/min (ΔA/min), substracting each reading from the previous one and averaging these values. Use this mean for calculations.

II- MICROTECHNIQUE
In a cuvette at 30-37ºC, place:

Reconstituted Reagent A	1 ml
Pre-incubate 3-4 minutes. Then add:	
Sample	20 ul

Mix immediately and wait 3 minutes. Follow A-I procedure.

B) 25ºC

I- MACROTECHNIQUE
Since activity at this temperature is lower, use 100 ul sample to reach required sensitivity. Start recording readings 4 minutes after sample addition. Follow A-I procedure.

II- MICROTECHNIQUE
Follow the steps described in A-II, but using 50 ul sample.

CALCULATIONS

CK U/l = ΔA/min x factor

In each case the corresponding calculation factor should be used, as shown on the table below:

Temperature / Wavelength	30-37ºC	25ºC	
	I or II	I	II
340 nm	8,095	4,127	3,333
334 nm	8,252	4,207	3,398
366 nm	15,000	7,647	6,176

QUALITY CONTROL METHOD

Each time the test is performed, analyze two levels of a quality control material (**Standatrol S-E 2 niveles**) with known creatine kinase activity.

REFERENCE VALUES

Temperature	25ºC	30ºC	37ºC*
Men	up to 80 U/l	up to 130 U/l	up to 195 U/l
Women	up to 70 U/l	up to 110 U/l	up to 170 U/l

*Calculated

It is recommended that each laboratory establishes its own reference values.

SI SYSTEM UNITS CONVERSION

Creatine kinase (U/l) x 0.017 = Creatine kinase (ukat/l)

PROCEDURE LIMITATIONS

See Known Interfering Substances under SAMPLE.

PERFORMANCE

a) Reproducibility: simultaneously processing replicates of the same sample in the same day, were obtained:

Level	S.D.	C.V.
144.5 U/l	± 3.36 U/l	2.33 %
450.4 U/l	± 5.98 U/l	1.33 %

Performing the same assay on different days, were obtained:

Level	S.D.	C.V.
147.5 U/l	± 3.13 U/l	2.12 %
451.2 U/l	± 6.89 U/l	1.53 %

b) Detection limit: depends on the photometer used and the wavelength. In spectrophotometer with 1 cm optical length square cuvettes, for a ΔA/min of 0.001, the minimum detectable activity change will be of 8 U/l (at 340 nm and 30 or 37ºC).

c) Dynamic range: the useful reading range is extended up to 0.250 O.D. (340 nm) or 0.140 O.D. (366 nm). For higher values dilute the sample with saline solution, correcting the results accordingly.

PARAMETERS FOR AUTOANALYZERS

For programming instructions check the user manual of the autoanalyzer in use.

WIENER LAB. PROVIDES

CK-NAC UV AA:
- 3 x 20 ml (60 ml Buffer) (Cat. Nº 1271303).
- 3 x 20 ml (3 x 20 ml Buffer) (Cat. Nº 1009309).
- 10 x 20 ml (200 ml Buffer) (Cat. Nº 1271353).

CK-NAC UV unitest:
- 20 vials x 2.5 ml Reagent (Cat. Nº 1271351).

REFERENCES

- D.G.K.C. - Z. Klin. Chem. 10:281 (1972).
- S.S.C.C. - Scand. J. Clin. Lab. Invest. 33:291 (1974).
- I.F.C.C. - Clinica Chimica Acta 105:147 F (1980).
- I.F.C.C. - Ann. Biol. Clin. 44/4:419 (1986).
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 4th ed., 2001.

SYMBOLS

The following symbols are used in packaging for Wiener lab. diagnostic reagents kits.

This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices

EC REP Authorized representative in the European Community

IVD "In vitro" diagnostic medical device

Contains sufficient for <n> tests

Use by

Temperature limitation (store at)

Do not freeze

Biological risks

Volume after reconstitution

Cont. Contents

LOT Batch code

Manufactured by:

Harmful

Corrosive / Caustic

Irritant

Consult instructions for use

Calibr. Calibrator

CONTROL Control

CONTROL + Positive Control

CONTROL − Negative Control

REF Catalog number

Wiener Laboratorios S.A.I.C.
Riobamba 2944
2000 - Rosario - Argentina
http://www.wiener-lab.com.ar
Dir. Téc.: Viviana E. Cétola
Bioquímica
A.N.M.A.T. Registered product
CK-NAC UV unitest:
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CK-NAC UV AA:
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Wiener lab.

2000 Rosario - Argentina