LIQUID LINE

CK-NAC UV AA

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

SUMMARY

Creatine Kinase (CK) is an intracellular enzyme mostly found in skeletal and heart muscles, as well as in 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:

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

$$\text{ATP} + \text{glucose} \xrightarrow{\text{hexoquinase}} \text{ADP} + \text{glucose-6-P}$$

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

In the reaction system, N-Acetyl-cysteine (NAC) works as activator of the Creatine Kinase, recommended by the IFCC.

PROVIDED REAGENTS

A. Reagent A: imidazole buffer solution.
B. Reagent B: compound solution containing creatine phosphate and reactive components 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 (G-6-PDH)	≥ 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

Provided Reagents: ready to use. These may be used separately as monoreagent, mixing 5 parts Reagent A with 1 part Reagent B (e.g.: 5 ml Reagent A + 1 ml Reagent B).

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 at 2-10°C until the expiration date stated on the box.
Once opened, they should not be maintained out of the refrigerator for extended periods of time. Avoid contamination.
(Premixed) Monoreagent: stable at 2-10°C for up to 20 days from preparation date.

INSTABILITY OR DETERIORATION OF REAGENTS

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

SAMPLE

Serum or heparinized plasma
a) Collection: obtain in the usual way.
b) Additives: heparin as anticoagulant.
c) Known interfering substances: no interferences have been observed from bilirubin up to 390 mg/l (39 mg/dl), triglycerides up to 12.5 g/l (1250 mg/dl), nor hemoglobin up to 0.15 g/dl (150 mg/dl).
See Young, D.S. under 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 at 2-10°C.

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 37°C. See the REFERENCE VALUES corresponding to each temperature.
- Reaction time: varies according to the selected procedure.

PROCEDURE

MONOREAGENT TECHNIQUE
Set instruments to zero with distilled water.

A) 30 - 37°C

In a cuvette at 30-37°C, place:

Monoreagent	1 ml
Pre-incubate 3-4 minutes. Then add:	
Sample	40 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.

B) 25°C

Follow the procedure indicated A) procedure but using 80 ul Sample and waiting for 4 minutes after the addition.

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	30-37°C	25°C
340 nm	4,127	2,142
334 nm	4,207	2,183
366 nm	7,429	3,856

QUALITY CONTROL METHOD

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

REFERENCE VALUES

Temperature	25°C	30°C	37°C[1]
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

[1]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: CLSI's protocol EP15-A was applied. Two activity levels were analyzed in replicates of four, during 5 days. With the obtained results total and intra-assay precision were calculated.

Intra-assay precision (n = 20)

Level	S.D.	C.V.
162 U/l	± 1.08 U/l	0.66 %
362 U/l	± 1.61 U/l	0.44 %

Total precision (n = 20)

Level	S.D.	C.V.
163 U/l	± 4.10 U/l	2.51 %
356 U/l	± 6.72 U/l	1.89 %

b) Linearity: usually, the reaction is linear up to 0.130 O.D. ΔA/min (approximately 550 U/l). For higher values, dilute the sample in 1:2 or 1:5 with saline solution and repeat the determination observing the same assay conditions and multiplying the obtained result by the dilution performed. In autoanalyzers, linearity up to 1800 U/l may be observed.

c) Analytical sensitivity: depends on the photometer used and wavelength. In spectrophotometer at 340 nm with 1 cm optical length square cuvettes, for ΔA/min of 0.001, the minimum detectable activity change will be of 8 U/l.

PARAMETERS FOR AUTOANALYZERS

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

WIENER LAB. PROVIDES

120 ml: 5 x 20 ml Reagent A
1 x 20 ml Reagent B
(Cat. 1271360)

120 ml: 5 x 20 ml Reagent A
1 x 20 ml Reagent B
(Cat. 1009331)

120 ml: 5 x 20 ml Reagent A
1 x 20 ml Reagent B
(Cat. 1009251)

240 ml: 4 x 50 ml Reagent A
2 x 20 ml Reagent B
(Cat. 1009609)

REFERENCES

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- NCCLS document "Evaluation of the Linearity of Quantitative Analytical Methods", EP6-P, (1986).
- CLSI: Clinical and Laboratory Standards Institute (ex-NCCLS) - Protocols EP 15A, 2001 / EP 17A, 2004.

Symbols

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

CE — 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

Σ — 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

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