CREATINE KINASE

NAC. Kinetic UV

Quantitative determination of creatine kinase (CK).

CE IVD

PACKAGING

Ref.: 101-0452	Cont.: 20 x 10 mL
Ref.: 101-0209	Cont.: 4 x 50 mL

Store at 2 - 8ºC.

CLINICAL SIGNIFICANCE

Creatine kinase is a cellular enzyme with wide tissue distribution in the body. Its physiological role is associated with adenosine triphosphate (ATP) generation for contractile or transport systems.
Elevated CK values are observed in diseases of skeletal muscle and after myocardial infarction[1,5,6].
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE OF THE METHOD

Creatine kinase (CK) catalyses the reversible transfer of a phosphate group from phosphocreatine to ADP. This reaction is coupled to those catalysed by hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6P-DH):

$$\text{Phosphocreatine + ADP} \xrightarrow{\text{CK}} \text{Creatine + ATP}$$

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

$$\text{G6P + NADP}^+ \xrightarrow{\text{G6P-DH}} \text{6-Phosphogluconate + NADPH + H}^+$$

The rate of NADPH formation, measured photometrically, is proportional to the catalytic concentration of CK present in the sample[1,2].

REAGENTS

R 1 Buffer	Imidazol pH 7.0	100 mmol/L
	Glucose	20 mmol/L
	Magnesium acetate	10 mmol/L
	EDTA	2 mmol/L
R 2 Substrate	ADP	2 mmol/L
	AMP	5 mmol/L
	di-Adenosine-5- pentaphosphate	10 mmol/L
	$NADP^+$	2 mmol/L
	Hexoquinase (HK)	2500 U/L
	Glucose-6-phosphate dehydrogenase (G6P-DH)	1500 U/L
	N-acetyl cysteine	20 mmol/L
	Creatine phosphate	30 mmol/L

PRECAUTIONS
R1: H360- May damage fertility or the unborn child.
Follow the precautionary statements given in MSDS and label of the product.

PREPARATION

Working reagent (WR):
101-0452: Dissolve 1 tablet of R 2 Substrate with one vial of R 1.
101-0209: Dissolve the content R 2 Substrate in one vial of R 1.
Cap vial and mix gently to dissolve contents.
Stability: 5 days at 2-8º C or 24 hours at room temperature (15-25º C).

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8º C, protected from light and contaminations prevented during their use.
Do not use the tablets if appears broken.
Do not use reagents over the expiration date.
Signs of reagent deterioration
- Presence of particles and turbidity.
- Blank absorbance (A) at 340 nm ≥ 1.60.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 340 nm.
- Thermostatic bath at 25º C, 30º C or 37º C (± 0.1º C).
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

Serum or plasma[1]: Stability 7 days at 2-8º C, protected from light.
The creatin kinase activity decreases 10% after 1 day at 2-5º C or after 1 hour at 15-25º C.

PROCEDURE

Notes: CHRONOLAB SYSTEMS has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

1. Assay conditions:
   Wavelength: . . . . . 340 nm
   Cuvette: . . . . . .1 cm light path
   Constant temperature . . . . . .25º C / 30º C / 37º C
2. Adjust the instrument to zero with distilled water or air.
3. Pipette into a cuvette:

	25 - 30º C	37º C
WR (mL)	1.0	1.0
Sample (µL)	40	20

4. Mix, incubate for 2 minutes.
5. Read initial absorbance (A) of the sample, start the stopwatch and read absorbances at 1 minute intervals thereafter for 3 minutes.
6. Calculate the difference between absorbances and the average absorbance differences per minute (ΔA/min)

CALCULATIONS

25º- 30º C ΔA / min x 4127 = U/L CK
37º C ΔA / min x 8095 = U/L CK

Units: One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute, in standard conditions. The concentration is expressed in units per litre of sample (U/L).

Temperature conversion factors
To correct results to other temperatures multiply by:

Assay temperature	Conversion factor to		
	25º C	30º C	37º C
25º C	1.00	1.56	2.44
30º C	0.64	1.00	1.56
37º C	0.41	0.63	1.00

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures.

If control values are found outside the defined range, check the instrument, reagents and technique for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[I]

	25º C	30º C	37º C
Men, up to	80 U/L	130 U/L	195 U/L
Women, up to	70 U/L	110 U/L	170 U/L

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From detection limit of 0,878 U/L to linearity limit of 1300 U/L.
If the results obtained were greater than linearity limit, dilute the sample 1/10 with NaCl 9 g/L and multiply the result by 10.
Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (U/L)	144	478	146	494
SD	3,88	6,98	4,55	9,57
CV (%)	2,69	1,49	3,11	1,94

Sensitivity: 1 U/L = 0.0001 ΔA/min.

Accuracy: Results obtained using CHRONOLAB reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results obtained using 50 samples were the following:
Correlation coefficient $(r)^2$: 0,997.
Regression equation: y= 1,031x – 0,5355.
The results of the performance characteristics depend on the analyzer used.

Interferences
No interferences were observed with bilirubin up to < 20 mg/dL and hemoglobin up to 10 g/L[1,2]. A list of drugs and other interfering substances with CK determination has been reported by Young et. al[3,4].

BIBLIOGRAPHY

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