CK (NAC act.) KIT

(Mod. IFCC method)

For the determination of CK activity in serum.

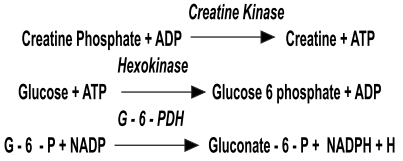
(For Invitro Diagnostic Use Only)

Summary

CK is mainly found in all muscle and brain tissue. It plays an important role in the energy storing mechanism of the tissues. Increased levels are found in myocardial infarction, cerebrovascular diseases, muscular dystrophy, pulmonary infarction &, electrical shocks. Increased levels can also be caused by intra muscular injections,strenuous exercise and recent surgery. Early pregnancy may produce decreased levels.

Principle

Creatine Kinase catalyse the reaction between Creatinine Phosphate and ADP to form creatine and ATP. The ATP formed along with Glucose is catalysed by Hexokinase to form Glucose 6 phosphate. The Glucose 6 phosphate reduces NADP to NADPH in the presence of Glucose 6 phosphate dehydrogenase. The rate of reduction of NADP to NADPH is measured as an increase in absorbance which is proportional to the CK activity in the sample.



Normal reference values

Serum (male) : 24 - 195 U/L at 37°C

(female) : 24 - 170 U/L at 37°C

It is recommended that each laboratory establish its own normal range representing its patient population.

Contents	**2 x 10 ml**	**2 x 25 ml**	**2 x 75 ml**
L1 : Enzyme Reagent	2 x 8 ml	2 x 20 ml	2 x 60 ml
L2 : Starter Reagent	2 x 2 ml	2 x 5 ml	2 x 15 ml

Storage / stability

Contents are stable at 2-8°C till the expiry mentioned on the labels.

Reagent Preparation

Reagents are ready to use.

Working reagent : For sample start assays a single reagent is required. Pour the contents of 1 bottle of L2 (Starter Reagent) into 1 bottle of L1 (Enzyme Reagent). This working reagent is stable for at least 10 days when stored at 2-8°C.

Alternatively for flexibility as much of working reagent may Alternatively 0.8 ml of L1 and 0.2 ml of L2 may also be used instead of 1 ml of the working reagent directly during the assay.

Sample material

Serum. Free from hemolysis. CK is reported to be stable in serum for 3 days at 2-8°C.

Procedure

Wavelength / filter : 340 nm

Temperature : 37°C / 30°C / 25°C

Light path : 1 cm

Substrate Start Assay :

Pipette into a clean dry test tube labelled as Test (T) :

Addition Sequence	**(T) 25°C / 30°C**	**(T) 37°C**
Enzyme Reagent (L1)	0.8 ml	0.8 ml
Sample	0.05 ml	0.02 ml
Incubate at the assay temperature for 1 minute and add		
Starter Reagent (L2)	0.2 ml	0.2 ml

Mix well and read the initial absorbance A_0 & repeat the absorbance reading after every 1, 2, & 3 minutes. Calculate the mean absorbance change per minute (ΔA/ min.)

Sample Start Assay :

Pipette into a clean dry test tube labelled as Test (T) :

Addition Sequence	**(T) 25°C / 30°C**	**(T) 37°C**
Working Reagent	1.0 ml	1.0 ml
Incubate at the assay temperature for 1 minute and add		
Sample	0.05 ml	0.02 ml

Mix well and read the initial absorbance A_0 after 1minute & repeat the absorbance reading after every 1, 2, & 3 minutes. Calculate the mean absorbance change per minute (ΔA / min.)

Calculations

Substrate / Sample start

CK Activity in U/L 25°C / 30°C = ΔA/ min. x 3333

37°C = ΔA / min. x 8095

TEMPERATURE CONVERSION FACTORS

Assay Temperature	**Desired Reporting Temperature**		
	25°C	**30°C**	**37°C**
25°C	1.00	1.56	2.44
30°C	0.64	1.00	1.56
37°C	0.41	0.63	1.00

Linearity

The procedure is linear upto 2000 U/L at 37°C. If the absorbance change (ΔA / min.) exceeds 0.250, use only the value of the first two minutes to calculate the result, or dilute the sample 1+ 9 with normal saline (NaCl 0.9%) and repeat the assay (Results x 10).

Note

Samples having a high activity show a very high initial absorbance as most of the NADP is converted prior to the start of measurement. If this is suspected then dilute the sample and repeat the assay.

The working reagent or the combined reagent should have an absorbance below 0.800 against distilled water at 340 nm. Discard the reagent if the absorbance is above 0.800.

References

IFCC methods for the measurement of catalytic concentrations of enzymes, JIFCC. (1989) 1 : 130

System Parameters

Reaction	:	U.V. Kinetic
Wavelength	:	340 nm
Zero Setting	:	Distilled water
Incub. Temp.	:	37°C
Incub. Time	:	---
Delay Time	:	60 sec.
Read Time	:	180 sec.
No. of read.	:	4
Interval	:	60 sec.
Sample Vol.	:	0.02 ml
Reagent Vol.	:	1.00 ml
Standard	:	---
Factor	:	8095
React. Slope	:	Increasing
Linearity	:	2000 U/L
Units	:	U/L

CKN(Kin):02(P)

Coral Clinical Systems

Gitanjali, Dr. Antonio Do Rego Bagh,
Bambolim Complex P.O. GOA - 403 202, INDIA