CK-NAC

ENZOPAK

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(KINETIC IFCC METHOD)

Reagent kit for quantitative estimation of creatine kinase in serum.

BACKGROUND AND SYNOPSIS :

The estimation of creatine kinase activity using creatine phosphate rather than creatine as substrate was first used by Oliver and later modified by Rosalki. Szasz further determined optimal test conditions for the method.

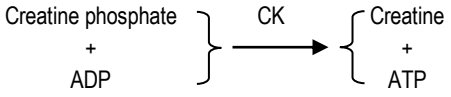
ENZOPAK CK-NAC is based upon the modification of Szasz method. This method has the advantages of being a more sensitive method, as a reverse reaction is faster and hence requires less sample volume.

Creatine kinase looses its activity due to oxidation of sulfhydryl groups at the active site of the enzyme. By addition of thiol compounds, reactivation of the enzyme can occur. N-acety-L-cysteine (NAC) is an activator of choice for this system. Interference by myokinase is eliminated by using AMP (Adenosine Monophosphate) and DAPP (Diadenosine 5' penta phosphate) which inhibit the enzyme.

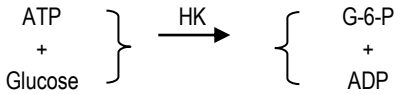
ENZOPAK-CK-NAC is according to International Federation of Clinical Chemistry (IFCC) and offers the advantage of a sensitive and specific test system.

PRINCIPLE :

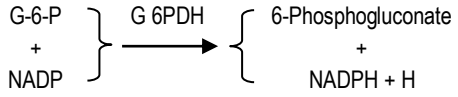
A) In this reaction, Creatine Kinase catalyzes the formation of ATP from Creatine Phosphate and ADP.



B) Glucose is converted to Glucose-6-Phosphate by Hexokinase using ATP as a source for PO_4 moiety.



C) Glucose-6-phosphate is oxidized by G-6PDH to 6-phosphogluconate reducing NADP to NADPH. The reaction after the lag phase is monitored by the increase in absorbance at 340 nm and is directly proportional to the creatine kinase activity. (i.e. the formation of NADPH is in equimolar amount as that of formation of creatine.)



CK	=	Creatine Kinase
HK	=	Hexokinase
G6P	=	Glucose-6-phosphate
G6PDH	=	Glucose-6-phosphate dehydrogenase

N-acetylcysteine acts as a thiol activator and DAPP & AMP inhibit the interfering myokinase activity.

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DIAGNOSTIC SIGNIFICANCE :

Creatine kinase is found in cardiac muscles, skeletal muscles and cerebral tissues. Consequently, damage or disease (e.g. myocardial infarction, acute cerebrovascular disease, muscular dystrophy or injury) of these tissues will result in elevated serum CK levels. In the case of myocardial infarction, CK activity begins to rise within 4 to 6 hours, peaks between 18 to 30 hours and returns to normal by the third day. Marginally increased levels may be found due to severe exercise and by large multiple intramuscular injections.

With other symptoms and suggestive history, serum CK estimation is an important parameter of choice for myocardial infarction and follow up.

REAGENT COMPOSITION

Active Ingredients	Concentration
Reagent-1	
* ADP	1.5mmol/L
* Hexokinase	$\geq$ 2000 U/L
* G6 PDH	$\geq$ 1.5 U/L
* NADP	1.2 mmol/L
* NAC	10 mmol/L
* CP	20 mmol/L
* AMP	2.5 mmol/L
Reagent-2	
*Buffer	100 mmol/L
*Detergent	0.5 mmol/L

pH 6.8$\pm$ 0.1 at 25^0 C

Also contains non-reactive fillers and Stabilizers.

PRESENTATION :

	NO. of Bottles/Vials		
All reagents to be stored at 2-8^0C	15 x 1.1 ml	10 x 3 ml	10 x 11 ml
1. CK-NAC (ENZYMES, ACTIVATOR)	15	10	10
2. CK-NAC (BUFFER)	1	1	10

PRECAUTION :

ENZOPAK CK-NAC is for *IN-VITRO* diagnostic use only. Use automated pipettes for better accuracy.

Reagent contains Sodium Azide, DO NOT INGEST.

PREPARATION OF WORKING REAGENT :

FOR 15 x 1.1 ml.

Add 1.1 ml. of 2 CK-NAC to one bottle of 1 CK-NAC. Mix to dissolve. Use after 10 minutes. The working reagent is stable for 21 days at 2-8^0C.

FOR 10 x 3 ml.

Dissolve the contents of one bottle of 1 CK-NAC with 3 ml. of 2 CK-NAC (Buffer). Mix well. Use after 10 minutes. Store at 2-8^0C, when not in use. The working reagent is stable for 21 days at 2-8^0C.

FOR 10 x 11 ml.

Dissolve the contents of one bottle of 1 CK-NAC with 11 ml. of 2 CK-NAC (Buffer). Mix well. Use after 10 minutes. Store at 2-8^0C, when not in use. The working reagent is stable for 21 days at 2-8^0C.

REAGENT STORAGE & STABILITY :

ENZOPAK CK-NAC reagents are stable untill the expiry date stated on the label.

SPECIMEN COLLECTION :

Fresh, clear serum under fasting condition with no hemolysis is the specimen of choice. Plasma collected with heparin as an anticoagulant may also be used.

REACTION PARAMETERS :

- Type of Reaction : Kinetic/Increasing OD
- Wavelength : 340 nm
- Flowcell Temperature : 30 ^{0}C or 37 ^{0}C
- Delay Time : 120 Seconds
- Interval Time : 30 Seconds
- No. of readings : 4
- Sample Volume : 50 Microlitres (0.05 ml)
- Working Reagent Volume : 1.0 ml.
- Factor : 3376
- Light Path : 1.0 cm.
- Zero setting with : Distilled Water

PROCEDURE :

For laboratories using instruments with 1.0 ml. cuvette capacity.

PIPETTE INTO TEST TUBES	TEST	REACTION TEMP.
• WORKING REAGENT (ml)	1.00	Use either 37^{0}C / 30^{0}C
• SAMPLE (ml)	0.05	

Mix immediately and read first absorbance of test at 120 seconds then second, third and fourth absorbance at an interval of 30 seconds at 340 nm. Determine the mean change in absorbance per minute (ΔA/ min.)

NOTE :

For laboratory using instruments with cuvette capacity more than 1.0 ml. increase sample and working reagent volumes proportionally.

TEST RESULTS :

CK Activity (IU/L) = F X ΔA/minute

Where F = 3376 (based on the millimolar absorption of NADPH at 340 nm.)

NORMAL VALUES :

	At 30^{0}C	At 37^{0}C
MEN :	15-130 IU/L	25-200 IU/L
WOMEN :	15-110 IU/L	25-170 IU/L

LINEARITY :

The method is linear upto 1000 IU/L For the sample values higher than 1000 IU/L, dilute the sample suitably with 0.9 % saline and repeat the assay. Obtain test results by applying proper dilution factor.

REFERENCES :

- OLIVERS, I.T. Biochem J., 61:116 (1985)
- ROSALKI, S.B. J.lab Clin. Med. 69.696 (1967)
- TIETZ N., (ed). Fundamentals of Clinical Chemistry. W.B. Saunders Co., Philadelphia PA 1976.

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