ANA BIO ISP CK - Nac

(IFCC Method)

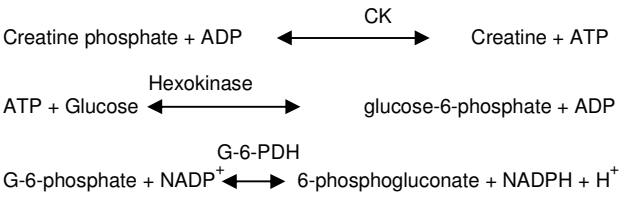
For Miura Instruments

Intended Use

CK- NAC is a reagent set for determination of creatine kinase activity in serum and plasma based on UV-Kinetic method.

Principle

Creatine Kinase catalyzes the conversion of Creatine phosphate and ADP to creatine and ATP. ATP phosphorylates glucose to glucose-6-phosphate in the presence of hexokinase.Glucose-6-phosphate is oxidized to 6-phosphogluconate, reducing NADP to NADPH in presence of G-6-PDH. The rate of increase in NADPH absorbance at 340nm is directly proportional to the activity of creatine kinase in serum/plasma.



Components & Concentration of Reagents

Reagent	Component	Concentration
Reagent 1	Imidozole buffer pH 6.6	125 mmol/L
	HK	≥ 800 U/L
	Nac	25 mmol/L
	G6P-DH	≥ 800 U/L
	NADP	2.5 mmol/L
	Mg Acetate	10 mmol/L
	Stabilizers, excipients & surface active agents	
Reagent 2	CP	30 mmol/L
	ADP	2 mmol/L
	AMP	6 mmol/L
	DAPP	0.010 mmol/L
	Stabilizers, excipients & surface active agents	

Reagent storage and stability

The reagent kit should be stored at 2° - 8°C and is stable till the expiry date indicated on the label.
A slight variation in the composition of the components may occur between batches, but this has no effect on the test results.
After opening, the vial R1 and R2 are stable 30 days if recapped immediately and protect from contamination, evaporation, direct light and stored at correct temperature.

Specimen collection and preservation

Blood should be collected in a clean dry container. Although serum is preferred, plasma with heparin or EDTA can be used. The assay should be carried out as far as possible on the same day. Serum and plasma samples are stable for 1 week at 4°C and 1 month at -10°C. The samples should be brought to room temperature prior to use. Avoid use of haemolysed and grossly contaminated samples.

Automation

This kit, though developed and manufactured to be used as manual assay and with I.S.E. Miura Analyzer, can be used also with other analyzers able to meet the specifications indicated in section "Reaction conditions – Test procedure" Application sheets are available for automatic instruments.
All applications not explicitly approved by KDPL. Cannot be guaranteed in terms of performance, and must there be established by the operator.

Calibration

For Calibration use the "Multicalibrator"

Calibration Stability

For the instrumentation series Miura, the calibration is recommended to be done every 10 days.

Materials required but not supplied in the kit

Calibrators and controls

Assay guidelines for Analyzer I.S.E. Miura

Analyte Name	CKNac	
Method Code	CkNac	
Type	Kinetic-Substrate Start	
Unit	IU/L	
Filter F1	340 nm	
Blank in	Not Use	
Step	Reaction Volume	U.M.
Volume reagent R1	200	µl
Sample Volume	10	µl
Incubation R1, S → R2	300	Sec.
Volume reagent R2	50	µl
Final Incubation	120	Sec.
Kinetic reading	192	Sec.

Temperature Conversion:

Following factors can be used for conversion of IU/l from one temperature to another:

Assay Temperature	Desired temperature		
	25°C	30°C	37°C
25°C	1.00	1.53	2.38
30°C	0.65	1.00	1.56
37°C	0.42	0.64	1.00

Note: Since temperature conversion factors are given only as an approximate conversion, it is suggested that values be reported at the temperature of measurement

Normal Range

	at 25°C	at 30°C	at 37°C
MEN	**< 80 IU/l**	**< 130 IU/l**	**< 190 IU/l**
WOMEN	**< 70 IU/l**	**< 110 IU/l**	**< 165 IU/l**
BABIES (2 – 12 months)	**< 136 IU/l**	**< 210 IU/l**	**< 325 IU/l**
CHILDREN (Above 12 months)	**< 94 IU/l**	**< 150 IU/l**	**< 225 IU/l**

Note: Expected range varies from population to population and each laboratory should establish its own normal range.

Limitations

- Avoid using hemolysed serum since red blood cells may release enzymes and intermediates such as ATP and Glucose-6-phosphate which may interfere in the assay.
- CK activity in serum may be elevated in patients receiving intramuscular injections upto one week prior to sample collection. Strenuous and unusual physical exercise may also cause elevated CK activity.
- The working solution is considered unsatisfactory and should not be used the absorbance exceeds 0.700 at 340 nm against distilled water.
- If the CK activity exceeds 2000 IU/L; then dilute the specimen suitable with normal saline and repeat the assay. In such case, the results obtained should be multiplied with the dilution factor to obtain correct CK activity. The dilution of a sample that initially exceeds linearity often results in a higher than expected value; therefore instead of dilution it is recommended that a smaller sample volume be used.

Quality Control

To ensure adequate quality control measures, it is recommended that each batch should include a normal and an abnormal commercial reference control serum. It should be realized that the use of quality control material checks both instrument and reagent functions together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glassware, Wavelength setting, Expiration date of reagents and accuracy of prob aspiration.

Accuracy-Recovery
N= 60, R = 0.999, Y = 0.92x+7.89

Interference
Triglycerides below 1000 mg/dl does not interfere in the reaction. Haemoglobin interferes at concentrations above 50.0 g/L. Ascorbic Acid influences the reaction at concentrations over 100 mg/dl.

Precision of the Method

Within-run					
Range	U.M	Mean	S.D.	C.V.(%)	No. run
Low	IU/L	148	1.25	1.0	20
High	IU/L	250	1.5	0.8	20
Between run					
Range	U.M	Mean	S.D.	C.V.(%)	No. run
Low	IU/L	145	1.4	1.1	20
High	IU/L	250	2.0	0.6	20

Sensitivity
At 340 nm, the activity of CK-Nac of 3 IU/L can estimate.

References

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4. Rosano, T. G, Clayson, K.J. & Strandjord, P.E. **Clin. Chem**. 22, 1078 (1976).
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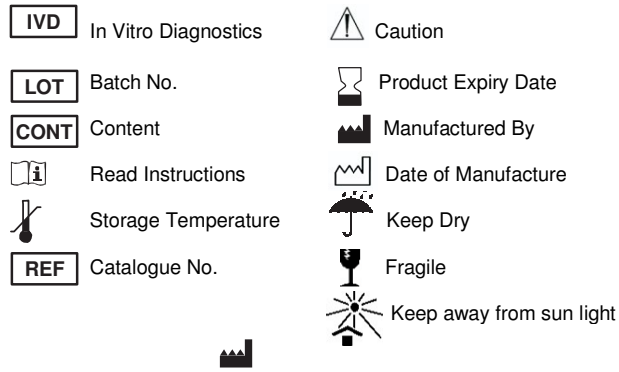
6. Scandinavian Society for Clinical Chemistry and Clinical Physiology, Scand **J. Clin. Lab Invest**. 37, 711 (1976).
7. Melattini F, Giannini G, and Tarli P., **Clin. Chem**.; 24, 3 (1978).
8. In-house test data,

Symbols



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