ANA BIO CLR CK - Nac

(UV-Kinetic method)

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.

$$\text{Creatine phosphate} + \text{ADP} \xrightarrow{\text{CK}} \text{Creatine} + \text{ATP} \xrightarrow{+\text{Glucose} + \text{Hexokinase}} \text{glucose-6-phosphate} + \text{ADP}$$

$$\text{G-6-phosphate} + \text{NADP}^{+} \xrightarrow{\text{G-6-PDH}} \text{6-phosphogluconate} + \text{NADPH} + \text{H}^{+}$$

Reagent provided

1. Reagent R1
2. Reagent R2

Working Reagent Preparation

Prepare working reagent by mixing Reagent R1 and Reagent R2 in the ratio 4:1 as per the number of tests required.

Reagent storage and stability

The reagent kit should be stored at 2-8℃ and is stable till the expiry date indicated on the label.
The working reagent (4 R1 + 1 R2) is stable for 10 days at 2-8℃.

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℃ and 1 month at -10℃. The samples should be brought to room temperature prior to use. Avoid use of haemolysed and grossly contaminated samples.

Assay guidelines for Analyzers

Reaction type	Kinetic Reaction
Reaction Slope	Increasing
Wavelength	340 nm
Flow cell Temperature	37℃
Delay time	180 seconds
Interval time	30 Seconds
No. of readings	4
Blank	Distilled water
Sample volume	40 µl (0.04 ml)
Working Reagent volume	1000 µl (1.0 ml)
Factor	4127
Linearity	Up to 2000 IU/l

Assay guidelines for Manual procedure

Pre warm the required amount of working reagent at 25℃ / 30℃ before use. Perform the assay as given below:

Reagents	**Standard**	**Test**
Working Reagent	1000 µl (1.0 ml)	1000 µl (1.0 ml)
Serum/Plasma	-	40 µl (0.04ml)

1. Mix thoroughly and transfer the assay mixture immediately to the thermo stated cuvette and start the stop watch simultaneously.
2. Record the first reading at 180th second and subsequently 4 more readings with 30 seconds interval at 340 nm.

Calculation

Activity of CK-NAC In IU/l = ΔAbs/min. x 4127

Temperature Conversion:

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

Assay Temperature	Desired temperature		
	25℃	30℃	37℃
25℃	1.00	1.53	2.38
30℃	0.65	1.00	1.56
37℃	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℃	at 30℃	at 37℃
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, 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, and cleanliness of glassware and accuracy of pipetting.

References

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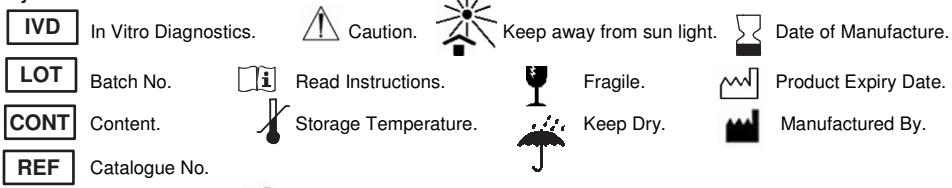
Symbols



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