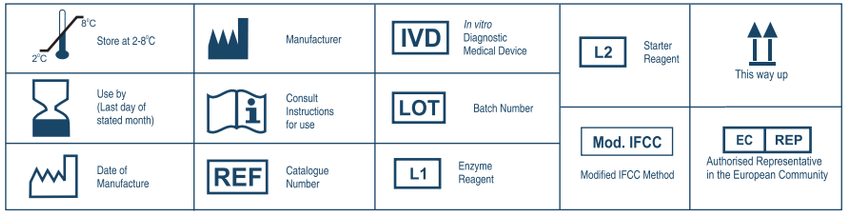
SBio CK (NAC act.) KIT

(Modified IFCC Method)

(For invitro diagnostic use only)

REF	90322225	90322275
Pack Size	2 x 25 ml	2 x 75 ml

CE



INTENDED USE

CK (NAC act.) Kit is used for the determination of Creatine Kinase (CK) Activity in Serum.

PRINCIPLE OF THE TEST

Creatine Kinase catalyses 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.

$$\text{Creatine Phosphate} + \text{ADP} \xrightarrow{\textit{Creatine Kinase}} \text{Creatine} + \text{ATP}$$

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

$$\text{G-6-P} + \text{NADP} \xrightarrow{\textit{G-6-PDH}} \text{Gluconate-6-P} + \text{NADPH} + \text{H}$$

CLINICAL SIGNIFICANCE

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.

PRESENTATION	2 x 25 ml	2 x 75 ml
L1 : Enzyme Reagent	2 x 20 ml	2 x 60 ml
L2 : Starter Reagent	2 x 5 ml	2 x 15 ml

COMPOSITION

Imidazole Buffer 100 mM; pH 6.6; Creatine Phosphate 30 mM; Glucose 20 mM; N-acetyl-L-cysteine 20 mM; Magnesium Acetate 10 mM; EDTA 2 mM; ADP 2 mM; NADP 2 mM; AMP 5 mM; DAPP 10 µM; G 6 PDH ≥ 2.0 KU/L; HK ≥ 2.5 KU/L, Non Reactive Stabilizers, Detergents and Preservatives.

STORAGE / STABILITY

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

SAMPLE REQUIRED

Serum, free from haemolysis is required.

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 be made as and when desired by mixing together 4 parts of L1 (Enzyme Reagent) and 1 part of L2 (Starter Reagent). 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 WASTE AND DISPOSAL

Do not reuse the reagent containers, bottles, caps or plugs due to the risks of contamination and the potential to compromise reagent performance.

This product requires the handling of human specimen. It is recommended that all human sourced material are considered potentially hazardous and are handled in accordance with the OSHA standard on blood borne pathogens.

Appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.

Handle specimen, solid and liquid waste and test components in accordance with local regulations and NCCLS guidelines M29, or other published biohazard safety guidelines.

PROCEDURE

Wavelength/filter	:	340 nm
Temperature	:	37°C / 30°C / 25°C
Light path	:	1 cm

MATERIALS REQUIRED BUT NOT PROVIDED

General laboratory instrumentation like Spectrophotometer/Analyzer, Thermostatic Cuvette holder, Cuvettes, Micropipettes, Test tubes, Waterbath, Stopwatch/Timer.

Substrate Start Assay:

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

Addition Sequence	(T) 25°C / 30°C	(T) 37°C
Enzyme Reagent (L 1)	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 (L 2)	0.2 ml	0.2 ml

Mix well and read the initial absorbance A_0 after 1 min. and 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 labeled 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 1 minuteand 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

QUALITY CONTROL

The following process is recommended for QC during the assay of CK (NAC act.). *Define and establish acceptable range for your laboratory.

1. Two levels of control (Normal and Abnormal) are to be run on a daily basis.
2. If QC results fall outside acceptance criteria, recalibration may be necessary.
3. Review QC results and run acceptance criteria following a change of reagent lot.

SPECIFIC PERFORMANCE CHARACTERISTICS

Linearity:

The procedure is linear upto 2000 U/L at 37°C. If the absorbance change (Δ A/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).

Limit of detection:

The limit of detection for CK (NAC act.) is 8 U/L.

Interferences:

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Precision:

Precision studies were performed with two controls using NCCLS protocol EP5-A. The results of the precision studies are shown below:

Sample	Within-run		Between-run		Total	
	Mean	CV%	Mean	CV%	Mean	CV%
Control 1	172.54	5.10	168.35	2.51	340.89	7.61
Control 2	476.78	3.06	479.01	1.11	955.79	4.17

Method comparison:

Comparative studies were done to compare our reagent with another commercial CK (NAC act.) Assay. No significant differences were observed. Details of the comparative studies are available on request.

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

REFERENCE RANGE

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*.

NOTE

CK is reported to be stable in serum for 3 days at 2-8°C. 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. The reagent may be used in several automated analyzers. Instructions are available on request. Do not use turbid, deteriorated or leaking reagents.

REFERENCES

1. IFCC methods for the measurement of catalytic concentrations of enzymes, JIFCC. (1989) 1 : 130.
2. Chemnitz G. et.al. (1979) Dtsch Med. Wschr 104:257.
3. Szasz G. and E.W. Busch (1979). Paper presented at 3rd Eur. Congr. Clin. Chem. Brighton/England, June 3-8 (abstract).

Mfd. for:

Singapore Biosciences PTE Ltd.

11 Yishun Street 51, # 04-23, The Criterion, Singapore 767971.

EC REP

CMC Medical Devices & Drugs S.L.,
C/ Horacio Lengo No. 18, CP 29006, Malaga, Spain.

CKN1(SBio)/M420/VER-02