Manual
Procedure

MEDICHEM MIDDLE EAST
Clinical Chemistry Reagents
Liquid Stable Reagents

Cat. No. 17270	R1	1	x	40	ml
For 50 tests	R2	1	x	10	ml

Creatine Kinase (CK)

UV kinetic method. Activated NAC

Based on IFCC recommendations

Liquid Reagents

Test principle

Kinetic determination of CK activity after reactivation by N-acetylcysteine, according to the following reactions:

Creatine phosphate + ADP $\xrightarrow{CK}$ Creatine + ATP

ATP+ D-Glucose $\xrightarrow{HK}$ ADP + D-Glucose-6-phosphate

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

Creatine kinase catalyzes the reversible phosphorylation of ADP, in the presence of creatine phosphate, to form ATP and creatine.
The auxiliary enzyme hexokinase (HK) catalyzes the phosphorylation of glucose by the ATP formed, to produce ADP and glucose-6-phosphate (G6P). The G6P is oxidized to 6-phosphogluconate with the concomitant production of NADPH. The rate of NADPH formation, measured at 340 nm, is directly proportional to serum CK activity.

G6PDH= Glucose-6-phosphate-dehydrogenase

Concentrations in the test

Reagent R1		
N-Acetylcystein (NAC)	30	mmol/L
ADP	5.88	mmol/L
NADP	3	mmol/L
AMP	7.2	mmol/L
Diadenosine (5´) pentaphosphate	10	µmol/L
G-6-PDH **(Glucose-6-phosphate-dehydrogenase)**	≥ 2800	U/L
Hexokinase	≥ 4000	U/L
Reagent R2		
D-Glucose	40	mmol/L
Magnesium acetate	15.3	mmol/L
Creatine phosphate	150	mmol/L
EDTA	2.6	mmol/L
Stabilizer, preservative		

Stability and preparation of working reagent

Reagent R1: *liquid*.
Reagent R2: *liquid*.
All reagents are stable up to expiry date given on the label when stored at +2 ➔ +8 °C.

Working Reagent: (4+1)
Mix **4** volumes of bottle R1 with **1** volume of bottle R2.
Avoid direct exposure to light.
Stability: 14 days at 2 - 8 °C.

note: Don't use if bacterial contamination is evident (turbidity), and the working reagent has an absorbance greater than 0.700 at 340 nm against water.

Specimen collection and handling

1. Serum is the specimen of choice. Rosalki has reported that plasma (Heparin or EDTA)may be used.
2. CK in serum is stable for 24 hours at 20 - 25°C, for 7days at 2 - 8°C, and for 1 month at - 20°C when protected against evaporation.
3. Strenuous exercise or physical activity can produce elevated levels of CK in serum.

Calibrator

MediCal U Cat. No. 15011

Quality control

Meditrol N Cat. No. 15171
Meditrol P Cat. No. 15181

Procedure

Wavelength	Hg 340 nm (334 - 365 nm)
Spectrophotometer	340 nm
Cuvette	1 cm light path
Temperature	37°C
Measurement	against air or distilled water
Reaction	kinetic – increase

Assay **Incubate Working Reagent at 37°C:**

Sample	20 µl
Working Reagent	1000 µl
Mix, incubate for 3 min. at 37°C. Read the change in the absorbance per 1 min. for 3 min. Determine the mean absorbance change per 1 min. (ΔA/min).	

Calculation

Creatine Kinase activity (U/L) = ΔA/min. x Factor

Factor

Wavelength	340 nm	334 nm	365 nm
Factor at 37°C	8095	8252	15000

Note: It is recommended that each laboratory (as per instrument performance) could make its own factor (F) by the use of a calibrator according to the following formula:

$$F = \frac{\text{Conc.}_{\text{Clibrator}}}{\Delta/\text{min}_{\text{Calibrator}}}$$

Note: If myocardial infarction is suspected but the values obtained are below the specified limits, a recent infarct may have occurred. In this cases, tests should be repeated after 4 hours.

Linearity

Up to 1000 U/L.
If the result exceeds 1000 U/L, repeat the test using diluted sample (1+2)
with sodium chloride solution (0.9 %) and multiply the result by 3.

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Creatine Kinase (CK) Activated NAC

Interferences

1. Certain drugs and medications may affect the activity of CK, see Young, et al.
2. Elevated levels of bilirubin 20 mg/dl and hemoglobin 500 mg/dl have been found to have negligible effect on this method.

Precautions

1. The reagents may be irritating to skin, flush skin with water if contacted.
2. This reagent contains sodium azide as a preservative. don't ingest. May react with lead and copper plumbing to form highly explosive metal azide. Upon disposal, flush with a large volume of water to prevent azide build up.

Reference range

1 d.		< 712	U/L
2 – 5 d.		< 652	U/L
6 d. – 6 mth.		< 295	U/L
7 – 12 mth.		< 203	U/L
1 – 3 yr.		< 228	U/L
4 – 6 yr.		< 149	U/L
7 – 12 yr.	women	< 154	U/L
	men	< 247	U/L
13 – 17 yr.	women	< 123	U/L
	men	< 270	U/L
Adults	women	< 145	U/L
	men	< 170	U/L
Adults (Myocardial infarction)	women	> 167	U/L
	men	> 190	U/L

References

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