



PRINCIPLE

Creatine Kinase (CK) catalyzes the phosphorylation reaction of ADP, in presence of creatine phosphate to form creatine and ATP. Produced ATP reacts with glucose in presence of hexokinase (HK), forming new ADP and glucose-6-phosphate (G-6-P) which in presence of glucose-6-phosphate dehydrogenase (G6-P-DH) produces 6-phosphogluconate. The catalytic concentration of creatine kinase (CK) is determined from the rate of NADPH measured at 340 nm, which is formed in the last reaction of the enzyme chain.

N-acetylcysteine (NAC) has function as CK activator.

Creatine Phosphate + ADP \rightarrow Creatine + ATP

ATP + Glucose \rightarrow ADP + G6P

G6P + NADP⁺ + H₂O \rightarrow Gluconate-6P + NADPH + H⁺

CLINICAL SIGNIFICANCE

Creatine Kinase enzyme (CK) is present in cardiac, cerebral and skeletal muscle tissue. An increase of its hematic activity could be associated to myocardial infarction, acute cerebrovascular forms, traumas or diseases in charge of the muscular system. After a myocardial infarction, the level of CK begins to grow up between the fourth and the sixth hour succeeding to the happening, reaching the maximum peak between the eighteenth and the thirtieth hour, returning then to the normal value during the third day.

SAMPLE COLLECTION & STORAGE

☑ Serum, Plasma anticoagulated with heparin.

☑ The activity of CK is stable 7 days at 2-8°C

PRECAUTIONS

- * CK NAC kit is for in vitro diagnostic use only.
- * Bring all reagents to room temperature before use.

KIT CONTENT & STORAGE

Reagent A (Buffer Reagent) 10 ml - 2 Bottles

Reagent B (Enzyme Reagent) 5 ml - 1 Bottle

All reagents are to be stored at 2-8°C and stable till expiry date mentioned.

REAGENT PREPARATION

For Single test: Mix 800 µl of Reagent A with 200 µl of Reagent B (8: 2 Ratio)

For multiple tests: Mix 4 volumes of Reagent A with 1 volume of Reagent B (4: 1 Ratio)

Working Reagent is stable for 30 days at 2-8°C or 5 days at 20-25°C, protected from light.

Discard the reagent if presents an absorbance over 0.800 at 340 nm against distilled water

SYSTEM PARAMETERS

| | |
|-----------------|-------------|
| Reaction Type | Kinetic |
| Wave Length | 340nm |
| Flow Cell Temp | 37°C |
| Working Reagent | 1.0 ml |
| Sample Volume | 40 µl |
| Factor | 4127 |
| Delay Time | 180 sec |
| Interval | 60sec |
| No of Readings | 3 |
| Blank | Dist. Water |
| Units | IU/L |

PROCEDURE

Pipette in a clean dry test tube

| | |
|-----------------|-------|
| Working Reagent | 1.0ml |
| Sample | 40µl |

Mix gently, incubate for 3 minutes (180 seconds) and read initial absorbance. Repeat the absorbance readings exactly after 1, 2 and 3 minutes against blank. Calculate $\Delta A/\text{min}$.

CALCULATIONS

Conc of Total CKMB in serum (IU/L) = $\Delta A/\text{min} \times 4127$

LINEARITY

This method is linear up to 1600 IU/L. Samples exceeding linearity should be diluted and reassayed. The result has to be multiplied by the dilution factor.

| Males | Females |
|-------------|-------------|
| 24-195 IU/L | 24-170 IU/L |

Due to variation in inter-laboratory assay conditions, instruments and demography, it is recommended that each laboratory should establish its own normal range.

Bibilography

1. Kaplan, LA. Pesce, A.J.: Clinical Chemistry, Mosby Ed. (1996)
2. Mathieu, M., etcoll., Ann. Biol, Clin, 40,87 (1982).
3. NCCLS Document, "Procedures for the collection of arterial blood specimens". Approved Standard, 3rd Ed. (1999)
4. EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/181EECEdition : 2007/07/04