

Reagent kit for determination of the creatine kinase activity in serum based upon IFCC, DGKC, SCE, SFBC, NVKC, ACB, SGKC recommendations.

Creatine kinase (CK) is an enzyme which is found primarily in skeletal muscle, cardiac muscle and brain tissue. Elevated levels of CK are associated with myocardial infarction, various muscle disorders and diseases such as progressive Duchenne-type muscular dystrophy. In myocardial infarction, peak CK levels occur 24 to 36 hours after onset of chest pain and depending on the extent of damage can reach more than 10 times normal levels.

Principle

The CK Reagent is a modification of the Szasz procedure. CK reversibly catalyzes the transfer of a phosphate group from creatine phosphate to adenosine triphosphate (ATP) as products. The ATP formed is used to produce glucose-6-phosphate and ADP from glucose. This reaction is catalyzed by hexokinase (HK) which requires magnesium ions for maximum activity. The glucose-6 phosphate is oxidized by the action of the enzyme glucose-6 phosphate dehydrogenase (G-6-PDH) with simultaneous reduction of the coenzyme nicotinamide adenine dinucleotide phosphate (NADP) to give NADPH and 6-phosphogluconate. The rate of increase of absorbance at 340/380 nm due to the formation of NADPH is directly proportional to the activity of the CK in the sample.



Reference values

Female: 24-170 U/l (0,40-2,83 µkat/l)

Male: 24-195 U/l (0,40-3,25 µkat/l)

It is recommended that each laboratory should assign its own normal range.

Reagent

1.Reagent (R1)

Imidazole buffer, pH=6.60 100 mmol/l

Magnesium acetate 10 mmol/l

2.Reagent (R2)

N-Acetylcysteine 20 mmol/l

ADP 2 mmol/l

AMP 5 mmol/l

NADP 2 mmol/l

D-Glucose 20 mmol/l

Diadenosine pentaphosphate 10 µmol/l

EDTA 2 mmol/l

Hexokinase ≥ 3500 U/l

Glucose-6-phosphate dehydrogenase ≥ 2000 U/l

Creatine-phosphate 30 mmol/l

Sample

Serum free of haemolysis.

PROCEDURE

Preparation of working reagent:

Dissolve the (R2) in buffer (R1).

Stability: at 20-25°C: 2 days
at 2-8° C: 2 weeks

If the absorbance of working reagent is higher than 1.2 at 334 nm the reagent can not be used.

Assay conditions

Wavelength: 340 (334-365) nm

Temperature: 37°C

Cuvette: 1 cm light path

Read against: distilled water

Method: kinetic (increasing)

Pipette into cuvette

| | |
|--------------------------|------|
| Working reagent | 1 ml |
| Sample or control | 20µl |

Mix and after a 2-minute incubation, measure the change of absorbance per minute (ΔA/min) during 3 minutes.

Calibration (37°C, IFCC method)

S1: Distilled water

S2: Roche C.F.A.S. (Calibrator for automated system) or

Randox Calibration Serum Level I or

Randox Calibration Serum Level II

Calibration frequency

Two point calibration is recommended:

- after reagent lot change,
- as required following quality control procedures.

Calculation using calibration

$$\frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times C_{\text{standard}} = C_{\text{sample}}$$

A = Absorbance

C = Concentration

Calculation using factor

Activity (U/l): ΔA/min x 9786 (340 nm); (µkat/l): ΔA/min x 163,1

Activity (U/l): ΔA/min x 8922 (334 nm); (µkat/l): ΔA/min x 148,7

Quality control

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

PERFORMANCES DATA

The following data were obtained using the Hitachi 717 analyzer (37°C).

Linearity

The test is linear up to 2000 U/l (33,33 µkat/l)

Sensitivity

It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used. Under manual conditions however, a change of 0.001 Abs units/min is equivalent to 8.922 U/l (0,15µkat/l) Creatine-kinase activity at 334 nm.

Precision

| Sample | Reproducibility | | |
|-----------|----------------------|-------|------|
| | Average activity U/l | SD | CV% |
| sample I | 163.98 | 5.90 | 3.60 |
| sample II | 472.92 | 16.57 | 3.50 |

Correlation

Comparative studies were done to compare our reagent with another commercial Creatine-kinase reagent.

The results from these studies are detailed below.

Correlation coefficient: r=0.9991

Linear regression: y (U/l)= 1.035x-4.299

(x= other commercial reagent, y= own reagent).

Specificity

Bilirubin 855µmol/l (50mg/dl), lipid 500mg/dl, glucose 55.5mmol/l (1000mg/dl) and ascorbic acid 2.84mmol/l (50mg/dl) don't interfere with the assay at the given levels.

NOTE

Do not use reagents after the expiry date stated on each reagent container label. Do not use products, test solutions and reagents described above for any purpose other than described herein.

For in vitro diagnostic use only.

The following symbols are used on labels

 For in vitro diagnostic use

 Use by (last day of the month)

 Temperature limitation

 Batch Code

 Code

Bibliography

Ann. Biol. Clin.: 40, 99 (1982)