INTENDED USE
Diagnostic reagent for quantitative in vitro determination of Creatine Kinase in human serum and plasma.

CLINICAL SIGNIFICANCE
Creatine Kinase (CK) is a dimeric enzyme occurring in four different forms: a mitochondrial isoform and the cytosolic isoforms CK-MM (muscle type), CK-BB (brain type) and CK-MB (myocardial type). The determination of CK and CK-isoenzyme activities is utilized in the diagnosis and monitoring of myocardial infarction and myopathies such as the progressive Duchenne muscular dystrophy. Following injury to the myocardium, as occurs with acute myocardial infarction, CK is released from the damaged myocardial cells. In early cases a rise in the CK activity can be found just 4 hours after an infarction, the CK-activities reaches a maximum after 12-24 hours and then falls back to the normal range after 3-4 days. Myocardial damage is very likely when the total CK activity is above 190 U/l, the CK-MB activity is above 24 U/l (37°C) and the CK-MB activity fraction exceeds 6% of the total.

The assay method using creatine phosphate and ADP was first described by Oliver, modified by Rosalki and further improved for optimal test conditions by Szasz. CK is rapidly inactivated by oxidation of the sulfhydryl groups in the active center. The enzyme can be reactivated by addition of N-acetyl cysteine (NAC). Interference by adenylate kinase is prevented by the addition of diadenosine pentaphosphate and AMP. Standardized methods for the determination for CK using the "reverse reaction" and activation by NAC were recommended by the German Society for Clinical chemistry (DGKC) and the International Federation of Clinical Chemistry (IFCC), in 1977 and 1990 respectively. This assay meets the recommendations of the IFCC and DGKC.

PRINCIPLE
Creatine phosphatase + ADP \(\rightarrow\) Creatine + ATP
ATP + Glucose \(\rightarrow\) Glucose-6-phosphate + ADP
Glucose-6-phosphate + NAD \(\rightarrow\) Glucuronate-6-P+ NADH+H⁺

The rate of absorbance change at 340 nm is directly proportional to Creatine kinase activity.

REAGENT COMPOSITION
R1
Imidazole buffer, pH 6.1 125 mmol/l
Glucose 25 mmol/l
Magnesium acetate 12.5 mmol/l
EDTA 2 mmol/l
N-acetylcysteine 25 mmol/l
NADP 2.4 mmol/l
Hexokinase > 6.8 U/ml
R2
ADP 15.2 mmol/l
D-glukoso-6-phosphate-dehydrogenase > 8.6 U/ml
Creatine phosphate 250 mmol/l
AMP 25 mmol/l
Diadenosine pentaphosphate 130 µmol/l

REAGENT PREPARATION
Reagents are liquid, ready to use.

STABILITY AND STORAGE
The unopened reagents are stable till the expiry date stated on the bottle and kit label when stored at 2–8°C.

Two reagents method – substrate start
Reagents are ready to use. After the first opening the vials, reagents are stable for 30 days at 2–8°C in the dark.

Monoreagent method – sample start
Mix 4 portion of reagent R1 with 1 portion of reagent R2.

SPECIMEN COLLECTION AND HANDLING
Use unhaemolytic serum or plasma. (EDTA, heparin)
It is recommended to follow NCCLS procedures (or similar standardized conditions).

QUALITY CONTROL
Calibration with the calibrator XL MULTICAL, Cat. No. XSYS0034 is recommended.

QUALITY DATA
For quality control ERBA NORM, Cat. No. BLT00080 and ERBA PATH, Cat. No. BLT00081 are recommended.

UNIT CONVERSION
UL x 0.017 = µkat/l

EXPECTED VALUES
At 37°C
Male: 46 – 171 UI
Female: 24 – 145 UI
Children: ≥ 1 month
Unbilical cord blood 175 – 402 U/l
Newborns 468 – 1200 U/l
≤ 5 days 195 – 700 U/l
< 6 months 41 – 330 U/l
< 6 months 24 – 229 U/l

It is recommended that each laboratory verify this range or derives reference interval for the population it serves.

PERFORMANCE DATA
Data obtained within this section is representative of performance on ERBA XL systems. Data obtained in your laboratory may differ from these values.

Limit of quantification: 10.4 U/l
Linearity: 1800 U/l
Measuring range: 10.4 – 1800 U/l

PRECISION

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<th>Mean (U/l)</th>
<th>SD (U/l)</th>
<th>CV (%)</th>
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<td>516.18</td>
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<td>156.18</td>
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<td>433.2</td>
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COMPARISON
A comparison between XL-Systems CK (y) and a commercially available test (x) using 40 samples gave following results:

\[ y = 1.028 x - 4.32 U/l \]
\[ r = 0.999 \]

INTERFERENCES
Following substances do not interfere: haemoglobin interferes, bilirubin up to 15 mg/dl, triglycerides up to 600 mg/dl.

WARNING AND PRECAUTIONS
For in vitro diagnostic use. To be handled by entitled and professionally educated person.

Reagents of the kit are not classified like dangerous but contain less than 0.1% sodium azide - classified as very toxic and dangerous substance for the environment.

WASTE MANAGEMENT
Please refer to local legal requirements.

ASSAY PROCEDURE

1. Work solution

2. Sample

3. Mix and incubate for 3 min. at 37°C. Then:

   Reagent 1 (buffer) 1,000 ml
   Sample 0.050 ml
   Mix and incubate for 3 min. at 37°C. Then measure the absorbance and at the same time start the stopwatch. Read the absorbance again exactly after 1, 2 and 3 minutes. Calculate the average 1 minute absorbance change (ΔA).

   Reagent 2 (substrate) 0.250 ml
   Mix and incubate for 3 min. at 37°C. Then measure the absorbance and at the same time start the stopwatch. Read the absorbance again exactly after 1, 2 and 3 minutes. Calculate the average 1 minute absorbance change (ΔA).

   Applications for automatic analysers will be supplied on request.

CALCULATION

1. \[ CK (U/l) = \frac{\Delta A_{	ext{max}}}{f} \times C_{\text{cal}} \]
   \[ \Delta A_{	ext{cal}} = \text{calibrator concentration} \]

2. Using factor:

   \[ CK (UI) = f \times \Delta A/\min \]
   \[ f = \text{factor} \]
   \[ f = 4127 \text{ (at 340 nm)} \]

Applications for automatic analysers are available on request.
REFERENCES

SYMBOLS USED ON LABELS

<table>
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QUALITY SYSTEM CERTIFIED
ISO 9001 ISO 13485
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