CREATINE KINASE

Cat. No.	Pack Name	Packaging (Content)
BLT00017	CK 100	R1: 4 x 20 ml, R2: 1 x 20 ml

EN

CE IVD

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 isoenzyme and the cytosolic isoenzymes 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 phosphate + ADP $\xrightarrow{CK}$ Creatine + ATP

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

Glucose-6-phosphate+ NAD $\xrightarrow{G6PDH}$ Gluconate-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	> 8.8 U/ml
Creatine phosphate	250 mmol/l
AMP	25 mmol/l
Diadenosine pentaphosphate	103 µmol/l

12000039

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.
Stability: 2 days at 20–25°C in the dark
14 days at 2–8°C in the dark

SPECIMEN COLLECTION AND HANDLING
Use unheamolytic serum or plasma (EDTA, heparin)
It is recommended to follow NCCLS procedures (or similar standardized conditions).
Stability: 1 week at 2–8°C
1 day at 15–25°C
Stability at –20 °C: 4 weeks in the dark
Discard contaminated specimens.

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

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

UNIT CONVERSION
U/l x 0.017 = µkat/l

EXPECTED VALUES [4]
At 37°C

Male:	46 – 171 U/l
Female:	24 – 145 U/l
Children: [10]	
Umbilical 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 contained 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

Intra-assay precision Within run (n=20)	Mean (U/l)	SD (U/l)	CV (%)
Sample 1	396.6	3.6	0.91
Sample 2	516.18	4.86	0.94

Inter-assay precision Run to run (n=20)	Mean (U/l)	SD (U/l)	CV (%)
Sample 1	156.18	4.32	2.77
Sample 2	433.2	14.82	3.42

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
Wavelength: 340 nm
Cuvette: 1 cm

Two reagents method – substrate start

Reagent 1 (buffer)	1.000 ml
Sample	0.050 ml

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

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).

Monoreagent method – sample start

Working solution	1.000 ml
Sample	0.040 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) = $\frac{\Delta A_{sam}}{\Delta A_{cal}} \times C_{cal}$ C_{cal} = calibrator concentration

2. Using factor:
CK (U/l) = f x ΔA/min
f = factor
f = 4127 (at 340 nm)

Applications for automatic analysers are available on request.

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N/10/13/A/INT *Date of revision: 28.11. 2013*

ASSAY PARAMETERS FOR PHOTOMETERS

Mode	Kinetic
Wavelength 1 (nm)	340
Sample Volume (µl)	40
Reagent Volume (µl)	1000
Lag Time (sec.)	120
Kinetic Interval (sec.)	60
No. of readings	3
Kinetic factor	4127
Reaction temperature (°C)	37
Reaction direction	Increasing
Normal Low (U/l)	46
Normal High (U/l)	145
Linearity Low (U/l)	10.4
Linearity High (U/l)	1800
Absorbance Limit (Max.)	0.3
Blank with	Water
Units	U/l

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SYMBOLS USED ON LABELS

REF Catalogue Number

Manufacturer

See Instruction for Use

LOT Lot Number

CE Mark - Device comply with the Directive 98/79/EC

Storage Temperature

Expiry Date

IVD In Vitro Diagnostics

CONT Content

QUALITY SYSTEM CERTIFIED
ISO 9001 ISO 13485

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