LIQUIZYME

CK-MB

(Immunoinhibition Method)

BEACON

Code	Product Name	Pack Size
LS015A	Liquizyme CK-MB	15 T
LS015B	Liquizyme CK-MB	25 ml
LS015D	Liquizyme CK-MB	100 ml

Intended Use

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

Clinical Significance

Creatine Kinase (CK) is a dimetic enzyme occuring in four different forms: amitochondrial 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 exceds 6% of total.

The assay method using creatine phosphate and ADP was first described by Oliver, modified by Rosalki and further improved for optimal test conditionsbySzasz. 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 adenhlate kinase is prevented by the addition of diadenosine pentaphosphate and AMP. Standardized methods for the determination for CK using the "referse 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

Specific antibodies against CK-M inhibit the complete CKMM activity and the CK-M subunit of CKMB. Only CK-B activity is measured.

$$\text{Creatine phosphate} + \text{ADP} \xrightarrow{\text{CKBB/CKMB}} \text{Creatine} + \text{ATP}$$

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

$$\text{Glucose-6-P} + \text{NADH} \xrightarrow{\text{G6PDH}} \text{Glucose-6-P} + \text{NADPH} + \text{H}^{+}$$

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

Reagent Composition

Reagent 1 : Enzyme Reagent

Imdazole	:	125 mmol/L
D-Glucose	:	25 mmol/L
Magnesium acetate	:	12.5 mmol/l
EDTA-NA Salt	:	2 mmol/l
N-acetylcysteine	:	25 mmol/l
NADP	:	2.4 mmol/l
Hexokinase	:	>6.8 U/ml

Anti-CK antibodies (goat) blocking capacity up to 2000 U/L CK-MM

Reagent 2 : Starter Reagent

ADP	:	>0.2 mmol/L
D-glucoso-6-phosphate -dehydrogenase	:	>10000
AMP	:	>5 mmol/l
Sodium Azide	:	>0.8 mmol/l

Reagent Preparation

Reagent is 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.

Working Reagent Preparation

Mix 4 portion of reagent R1 with 1 portion of reagent R2.

Specimen Collection And Handling

Use unheamolytic serum or plasma (heparin, EDTA)
It is recommended to follow NCCLS procedures (or similar standardized conditions).

Loss of activity:

After 24 h	:	at +2 – +8°C	< 10%
after 1 h	:	at +15 – +25°C	< 10%

Discard contaminated specimens.

Quality Control

It's recommended to run normal and abnormal control sera to validate reagent performance.

Expected Values
At +37°C
Male / Female : 0-25 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 Beacon system. Data obtained in your laboratory may differ from these values.

Limit of quantification : 4 U/L
Linearity : 1000 U/L
Measuring range : 4 – 1000 U/L

Precision

Intra-assay precision Within run (n=20)	Mean (U/L)	SD (U/L)	CV (%)
Sample 1	10.69	0.53	4.92
Sample 2	50.74	2.19	4.32

Inter-assay precision Run to run (n=20)	Mean (U/L)	SD (U/L)	CV (%)
Sample 1	16.12	0.79	4.91

Comparison
A comparison between Beacon CK-MB (y) and a commercially available test (x) using 20 samples gave following results :
y = 0.9947 x - 0.2528 U/L
r = 0.997

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

Waste Management
Please refer to local legal requirements.

Assay Procedure
Wavelength : 340 nm
Cuvette : 1 cm

Addition Sequence	Volume
Working Reagent	1000 µl
Sample	50 µl

Mix and read the initial absorbance A after 5 minute and repeat the absorbance reading after every 1, 2 and 3 minutes. Calculate the mean absorbance change per minute. (ΔA/min).

Calculation
Using factor:
CK-MB activity (U/L) = ΔA/min x 6666

Applications for automatic analysers are available on request.

Assay Parameters For Photometers

Mode	Kinetic
Wavelength 1 (nm)	340
Sample Volume (µl)	50
Working Reagent Volume (µl)	1000
Lag time (sec.)	300
Kinetic Interval (sec.).	60
No. of Interval	3
Kinetic Factor	6666
Reaction temp. (°C)	37
Reaction Direction	Increasing
Normal Low (U/L)	0
Normal High (U/L)	25
Linearity Low (U/L)	4
Linearity High (U/L)	1000
Blank with	Water
Unit	U/L

References

1. Henderson, A.R., Donald W.M., Enzymes, Tietz Fundamentals of Clinical Chemistry, 5th Ed., Burtis, C.. & Ashwood, E.R. (W.B. Saunders eds. Philadelphia USA), (2001), 352.

Symbols Used On Labels

REF	Catalogue Number		Manufacturer
	See Instruction for Use	LOT	Lot Number
CONT	Content		Storage Temperature
	Expiry Date	IVD	In Vitro Diagnostics

BEA/24/CKM/LS/IFU Ver-03
21/06/2025