BECKMAN COULTER CE

AU

Instructions For Use

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CK-MB
Creatine kinase-MB isoenzyme

REF OSR61155 2 x 22 mL R1-1, 2 x 4 mL R1-2, 2 x 6 mL R2

For *in vitro* diagnostic use only.

PRINCIPLE

INTENDED USE

Enzymatic immuno-inhibition test for the quantitative determination of the creatine kinase-MB isoenzyme (CK-MB) in human serum and plasma on Beckman Coulter AU analysers.

SUMMARY AND EXPLANATION

Reference[1,2,3]

Creatine kinase (CK) EC 2.7.3.2, a dimer composed of M-muscle and /or B-brain subunits which associate to form the isoenzymes CK-MM, CK-MB and CK-BB, catalyses the reversible phosphorylation of creatine by ATP. Measurements of CK are primarily used in the diagnosis and treatment of myocardial infarction as well as being the most sensitive indicator of muscle damage. CK is increased whenever there is necrosis or regeneration of muscle and is therefore elevated in most myopathies such as Duchenne-muscular dystrophy and in conditions associated with muscle necrosis such as rhabdomyolysis. Total CK can also be increased in diseases of the CNS such as Reye's Syndrome where a 70 fold increase in CK activity indicates the severity of the encephalopathy.

CK-BB predominates in the brain, prostate, gut, lung, kidney, bladder, uterus, liver, thyroid and the placenta. CK-MM predominates in skeletal and cardiac muscle. In healthy individuals the total serum activity consists mainly of CK-MM while the other CK isoenymes and variants are only present in trace amounts or are undetectable. CK-MB is present to varying degrees in heart muscle and also to a minor degree in skeletal muscle.

CK activity rises following myocardial damage, with a significant increase in both the CK-MM and CK-MB fractions. The proportional rise in the CK-MB fraction to some extent depends on the size of the myocardial damage and on a history of previous myocardial damage. Changes in the ratio of CK-MB to CK-MM may be used to diagnose a myocardial infarction (MI), the ratio reaching a peak within 1.5 hours post MI. The diagnostic sensitivity and specificity of total CK estimation for the diagnosis of an MI can be improved by determining the rate of increase ("slope") of CK on serial samples obtained on admission and at 4, 8 and 12 hours thereafter. A 50% incremental increase per hour over the time period differentiates between an acute MI and non-infarction with an overall efficiency of 94%.

For patients in need of an early diagnosis of a myocardial infarction a rapidly appearing biomarker such as CK-MB plus a biomarker that rises later e.g. cardiac troponin is recommended for confirmation of the diagnosis.

METHODOLOGY

Reference[4,5]

R1 contains an antibody which binds to the M subunit of CK in the serum sample thereby inhibiting the activity of the M subunit. The B subunit of the enzyme remains free to act on the substrate present in R2. CK reversibly catalyses the transfer of a phosphate group from creatine phosphate to adenosine diphosphate (ADP) to give creatine and adenosine triphosphate (ATP) as products. The ATP formed is used to produce glucose-6-phosphate and ADP from glucose. This reaction is catalysed by hexokinase (HK) which requires magnesium ions for maximum activity. The glucose-6-phosphate is oxidised by the action of the enzyme glucose-6-phosphate dehydrogenase (G6P-DH) 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 nm due to the formation of NADPH is directly proportional to the activity of CK-MB in the sample.

CHEMICAL REACTION SCHEME

Reference[5]

Creatine phosphate + ADP	CK	Creatine + ATP
ATP + Glucose	HK, Mg^{2+}	ADP + Glucose-6-phosphate (G-6-P)
G-6-P + $NADP^{+}$	G6P-DH	6-Phosphogluconate + NADPH + H^{+}

SPECIMEN

TYPE OF SPECIMEN

Serum is the recommended specimen. Lipaemic, haemolysed and strongly icteric samples should be avoided. Allow specimen to clot and remove serum from cells promptly to minimise haemolysis and contamination by adenylate kinase from the red cells.

CK/CK-MB is stable in serum, protected from light, for 7 days when stored at 2…8°C, for 2 days when stored at 20…25°C and up to 1 year when stored at -20°C.[5,6,7,8]

Heparinised plasma, free from haemolysis, can also be used. Plasma samples may occasionally produce unpredictable rate reactions resulting in false low results.[6] Plasma with EDTA, oxalate or citrate is not recommended.

REAGENTS

WARNING AND PRECAUTIONS

Exercise the normal precautions required for handling all laboratory reagents.

Dispose of all waste material in accordance with local guidelines.

This product contains material of animal origin. The product should be considered as potentially capable of transmitting infectious diseases.

REACTIVE INGREDIENTS

Final concentration of reactive ingredients

Imidazole buffer (pH 6.7)	100 mmol/L	Diadenosine-pentaphosphate	0.01 mmol/L
Hexokinase (HK)	≥ 4.0 kU/L	EDTA	2.0 mmol/L
NADP	2.0 mmol/L	Glucose	20 mmol/L
G6P-DH	≥ 2.8 kU/L	Creatine phosphate	30 mmol/L
ADP	2.0 mmol/L	N-Acetylcysteine	0.2 mmol/L
Mg-Acetate	10 mmol/L	Activator	26 mmol/L

AMP	5.0 mmol/L	Antibody to CK-M-subunit	Variable
		Preservative	

The concentrations of the reactive components of the reagents shown on the kit label are the actual concentrations in the individual R1/R2 vials. The reagent composition which is shown in the Instructions For Use is the final concentration of these components in the reaction cuvette after addition of R1, Sample, and R2.

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

GHS HAZARD CLASSIFICATION

CK-MB R1-1	DANGER	
		
	H316	Causes mild skin irritation.
	H360	May damage fertility or the unborn child.
	P201	Obtain special instructions before use.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P308+P313	IF exposed or concerned: Get medical advice/attention.
	P332+P313	If skin irritation occurs: Get medical advice/attention.
		Imidazole 0.1 - < 1%
CK-MB R1-2	DANGER	
		
	H316	Causes mild skin irritation.
	H360	May damage fertility or the unborn child.
	P201	Obtain special instructions before use.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P308+P313	IF exposed or concerned: Get medical advice/attention.
	P332+P313	If skin irritation occurs: Get medical advice/attention.
		Imidazole 0.1 - < 1%
		Thioglycerol 1 - 5%
CK-MB R2	WARNING	
		
	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P273	Avoid release to the environment.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%

SDS	Safety Data Sheet is available at beckmancoulter.com/techdocs

REAGENT PREPARATION

R1:

The entire contents of bottle R1-2 must be transferred into the entire volume of R1-1. Mix by gentle inversion before placing on board the instrument.

R2:

The reagent is ready for use and can be placed directly on board the instrument.

STORAGE AND STABILITY

The reagents are stable, unopened, up to the stated expiry date when stored at 2…8°C. Once open, reagents stored on board the instrument are stable for 30 days.

INDICATIONS OF DETERIORATION

Visible signs of microbial growth, turbidity, precipitate, or any change in reagent colour may indicate degradation and warrant discontinuance of use.

CALIBRATION

CALIBRATION INFORMATION

The test is run in MB-mode. To provide a robust approach to generate the analyser specific MB factor, it is recommended that 5 separate calibration events should be used. A fresh vial of calibrator, utilising CK-MB Calibrator Cat no ODR30034 in the AB calibration mode, should be used for each of these runs. When calculating the mean factor from the separate runs the data should be examined for obvious outliers which should be repeated and replaced. For the AU2700/AU5400 this procedure needs to be performed for each ring. Quality control procedures should be undertaken immediately following calibration in accordance with good laboratory practice.

Re-establishment of the analyser specific MB factor is recommended when a critical part of the analyser is replaced.

Reagent blank measurement is recommended when changing to a new lot of reagent.

Traceability: This method has been standardised against the CK_{total} IFCC Reference Method with addition of antibody, performed manually and calculated via molar absorption coefficient ε.

QUALITY CONTROL

CK-MB Control Level 1 ODR30035 and Level 2 ODR30036 or other control materials with values determined by this Beckman Coulter system may be used.

Each laboratory should establish its own control frequency.

Good laboratory practice suggests that controls be tested each day patient samples are tested and each time calibration is performed. Values obtained for the controls should fall within specified limits as defined by the user. If any trends or sudden shifts in values are detected, review all operating parameters.

Each laboratory should establish guidelines for corrective action to be taken if controls do not recover within the specified limits.

Please note that the recovery of non-Beckman Coulter controls may vary with reagent lots of immunoassay products, due to the use of non-human materials in the controls.

TESTING PROCEDURE(S)

Refer to the appropriate Beckman Coulter AU analyser User Guide/Instructions For Use (IFU) for analyser-specific assay instructions for the sample type as listed in the Intended Use statement.

CALCULATIONS

The Beckman Coulter analyzers automatically compute the CK-MB activity of each sample.

REPORTING RESULTS

REFERENCE INTERVALS

Adults (37°C) < 24 U/L (0.4 µkat/L)[2,9]

Myocardial infarction: the probability of myocardial damage is high when the following conditions are fulfilled.[2]

	U/L	µkat/L
1. CK total	> 250	> 4.17
2. CK-MB	> 24	> 0.4
3. CK-MB activity is between 6 and 25% of total CK		

If myocardial infarction is suspected and the values found are below the stated limits, the infarction may be fresh. In this case the determinations should be repeated after 4 hours with a fresh sample.

A fraction of less than 6% indicates skeletal muscle damage. A fraction above 25% may indicate the presence of Macro-CK and requires further clarification.[2]

Expected values may vary with age, sex, sample type, diet and geographical location. Each laboratory should verify the transferability of the expected values to its own population, and if necessary determine its own reference interval according to good laboratory practice. For diagnostic purposes, results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

PROCEDURAL NOTES

LIMITATIONS

Macro-CK is an atypical form of CK that is composed of immunoglobulin complexes of normal isoenzymes. It migrates electrophoretically between MM and MB and is found mainly in elderly women. It is of no clinical significance, but its presence may cause falsely elevated results. If Macro-CK contribution is suspected, its presence should be confirmed by electrophoresis.

For inhibition of adenylate kinase the recommended inhibitors AMP/Ap5A are included, but as the inhibition can never be completely 100% a residual activity could affect low CK-MB activity results.

The inhibition capacity of the anti-CK-MM antibody is >99.75% at a CK-MM concentration of 2,000 U/L and >99% at a CK-MM concentration of 8,000 U/L. In samples where the total CK activity exceeds 8,000 U/L, CK-MB should be measured using a pre-diluted sample to ensure adequate inhibition of CK-M.

INTERFERENCES

Results of studies conducted to evaluate the susceptibility of the method to interference were as follows:

Icterus:	Interference less than 10% up to 40 mg/dL or 684 µmol/L bilirubin
Lipemia:	Interference less than 15% up to 500 mg/dL Intralipid

In very rare cases, gammopathy, especially monoclonal IgM (Waldenström's macroglobulinemia) can cause unreliable results.

Refer to Young[10] for further information on interfering substances.

PERFORMANCE CHARACTERISTICS

PERFORMANCE CHARACTERISTICS

Data contained within this section is representative of performance on Beckman Coulter systems. Data obtained in your laboratory may differ from these values.

LINEARITY

The test is linear within an enzyme activity range of 10 – 2,000 U/L (0.17 – 33.33 µkat/L).

SENSITIVITY

The lowest detectable level on an AU640 analyser was calculated as 5 U/L.

The lowest detectable level represents the lowest measurable level of CK-MB that can be distinguished from zero. It is calculated as the absolute mean plus three standard deviations of 20 replicates of an analyte free sample.

METHODS COMPARISON

Patient serum samples were used to compare this CK-MB OSR61155 assay on the AU640 against another commercially available CK-MB assay. Results of linear regression analysis were as follows:

y = 1.061x + 2.207	r = 1.000	n = 103	Sample range = 12 – 1,862 U/L

PRECISION

The following data was obtained on an AU640 using 3 serum pools analysed over 20 days.

n = 80	Within-run		Total	
Mean U/L	SD	CV%	SD	CV%
17	0.69	4.03	0.86	5.05
86	0.65	0.75	0.99	1.15
194	1.04	0.54	1.76	0.90

ADDITIONAL INFORMATION

DxC 700 AU requires that each reagent application has a standard format of abbreviated Closed Test Name. This Closed Test Name is required to allow automated loading of the calibrator information for each application as part of the DxC 700 AU Closed System. Refer to the table below for the Closed Test Name assigned to each application for this assay.

Test Name	Description
CKM1N	CK-MB (Serum)

Setting Sheet Footnotes
User defined
* Values set for working in U/L. To work in SI units (µkat/L) divide by 60
§ For use in AB mode only, refer to leaflet for further instruction

REVISION HISTORY

Revised Interferences section.

Preceding version revision history

Added new languages

REFERENCES

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