CK-MB

UV-Method
MANUAL
RX MONZA

INTENDED USE

For the quantitative *in vitro* determination of CK-MB in serum and plasma. This product is suitable for manual use and on the RX **monza**.

Cat. No.

CK 1296	R1a.	CK NAC/CK-MB Buffer/Glucose	1 x 70 ml
19 x 2.5 ml	R1b.	Enzymes/Coenzymes/ Substrate/Antibody	19 x 2.5 ml
		CONTROL	1 x 2 ml

GTIN: 05055273201420

CLINICAL SIGNIFICANCE[1]

Creatine Kinase (CK) is internationally accepted as a sensitive and specific indicator of acute myocardial infarction (AMI). There are 3 iso-enzymes of CK, CK-MM, CK-MB and CK-BB. CK-BB is produced by the brain in very small insignificant amounts. CK-MM is produced by the skeletal and heart tissue and CK-MB is produced by the heart muscle. In the vast majority of cases, the CK activity rises within 6 hours of an acute infarction. After about 20 hours, maximum values are observed. The CK activity generally returns to normal between the fourth and fifth day post- infarction.

PRINCIPLE[3]

Immunoinhibition Assay: An antibody is incorporated in the CK reagent. This antibody will bind to and inhibit the activity of the M subunit of CK-MB. This means that only the activity of the B subunit in serum is measured. If the activity is multiplied by a factor of 2, it will give the activity of CK-MB in serum.

SAMPLE

Serum, heparinized or EDTA plasma.

REAGENT COMPOSITION

Contents		Concentrations in the Test
R1a.	**CK NAC/CK-MB Buffer/Glucose**	
	Imidazole Buffer	0.10 mol/l, pH 6.7
	Glucose	20 mmol/l
	Mg-Acetate	10 mmol/l
	EDTA	2.0 mmol/l
R1b.	**Enzymes/Coenzymes/Substrate/Antibody**	
	ADP	2.0 mmol/l
	AMP	5.0 mmol/l
	Diadenosine Pentaphosphate	10 µmol/l
	NADP	2.0 mmol/l
	HK	≥ 2.5 U/ml
	G-6-PDH	≥ 1.5 U/ml
	N-Acetylcysteine	20 mmol/l
	Creatine Phosphate	30 mmol/l
	Antibody to CK-M	
	CONTROL	

SAFETY PRECAUTIONS AND WARNINGS

For *in vitro* diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

Solution R1a contains Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.

Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents, flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.

Caution: CONTROL

Human source material from which this product has been derived has been tested at donor level for the Human Immunodeficiency Virus (HIV 1, HIV 2) antibody, Hepatitis B Surface Antigen (HbsAg), and Hepatitis C Virus (HCV) antibody and found to be NON-REACTIVE. FDA approved methods have been used to conduct these tests.
However, since no method can offer complete assurance as to the absence of infectious agents, this material and all patient samples should be handled as though capable of transmitting infectious diseases and disposed of accordingly.

Health and Safety Data Sheets are available on request.

Please dispose of all biological and chemical materials according to local guidelines.

The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

STABILITY AND PREPARATION OF REAGENTS

R1a. CK NAC/CK-MB Buffer/Glucose
Contents ready for use. Stable up to the expiry date when stored at +2 to +8°C.

R1b. Enzymes/Coenzymes/Substrate/Antibody
Reconstitute one vial of Enzymes/Coenzymes/ Substrate/Antibody R1b with 2.5 ml of CK NAC/CK-MB Buffer/Glucose R1a.
Stable for 21 days at +2 to +8°C and 3 days at +15 to +25°C.

CONTROL
Open one vial of control serum very carefully, avoiding any loss of material, and reconstitute in exactly **2 ml** of redistilled water. Close vial and leave to stand for 15 minutes, dissolving contents completely by swirling or rotating gently. CK-MB is stable in the serum for 5 days at +2 to + 8°C, 8 hours at +25°C and 4 weeks at –20°C when frozen once.

MATERIALS PROVIDED

CK NAC/CK-MB Buffer/Glucose
Enzymes/Coenzymes/Substrate/Antibody
Control Serum

MATERIALS REQUIRED BUT NOT PROVIDED

Randox CKMB Control (Cat. No. CK 1212)
Randox Tri-Level Cardiac Control (Cat. No. CQ 3100) (Level 2 and Level 3)
Randox CK-MB Calibration (Cat. No. CK 2393)

PROCEDURE NOTE[4]

Macro-CK is an atypical form of CK that is composed of Immunoglobulin complexes of normal Isoenzymes. It migrates 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.

PROCEDURE

Before carrying out the assay to measure the activity of CK-MB in serum, it is necessary to measure total CK activity using the NAC-activated method.

FOR RX MONZA

Select CK-MB in the Run Test screen and carry out a water blank as instructed.

Pipette into a test tube:

	Reagent Blank S0	Standard S1	Sample
Saline	50 µl	-	-
Standard	-	50 µl	-
Sample	-	-	50 µl
Reagent	500 µl	500 µl	500 µl

Mix and aspirate into the Rx Monza.

CALIBRATION FOR RX MONZA

The use of saline and Randox CKMB Calibrator is recommended for Calibration.

FOR MANUAL USE

Wavelength:	340 nm, Hg 334 nm or Hg 365 nm
Cuvette:	1 cm light path
Temperature:	25°C, 30°C and 37°C
Measurement:	against air

Pipette into cuvette:

	Semi-micro	Macro
Sample	0.04 ml	0.1 ml
Enzymes/Coenzymes/ Substrate/Antibody	1.0 ml	2.5 ml

Mix, and let stand at the appropriate temperature for 10 minutes. Then add to a cuvette, and read absorbance A_1. Read absorbance A_2 exactly 5 minutes later.

$$\Delta A = A_2 - A_1$$

MANUAL CALCULATION

Wavelength	CK-B (U/l)	CK-MB (U/l)
340 nm	*825 x ΔA	*1651 x ΔA
Hg 334 nm	*841 x ΔA	*1683 x ΔA
Hg 365 nm	*1486 x ΔA	*2972 x ΔA

The absorbance change per minute (ΔA/min) may also be measured and the mean of 5 measurements should be used in the calculation.

The factors used will then become:-

Wavelength	CK-B (U/l)	CK-MB (U/l)
ΔA 340 nm/min	*4127	*8254
ΔA Hg 334 nm/min	*4207	*8414
ΔA Hg 365 nm/min	*7429	*14858

*Factor should be determined using the CK-MB Calibrator for each new batch of reagent
Factor = ((Cal ABS - Blank ABS)/Cal Target)

QUALITY CONTROL

Randox CKMB Control or Randox Tri-level Cardiac Control (Level 2 and Level 3) are recommended for daily quality control. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check cleanliness of all equipment in use.
3. Check water, contaminants i.e. bacterial growth may contribute to inaccurate results.
4. Check reaction temperature.
5. Check expiry date of kit and contents.
6. Contact Randox Laboratories Customer Technical Services, Northern Ireland +44 (0) 28 94451070.

INTERFERENCE

Haemolysis interferes with the assay.

REFERENCE RANGE

Myocardial infarction(MI)

The likelihood of myocardial damage is high when the following 3 factors are present:-

		Temperature		
		25°C	30°C	37°C
1	CK_{men}	>80 U/l[1]	>130 U/l[2]	>195 U/l*
	CK_{women}	>70 U/l[1]	>110 U/l[2]	>170 U/l*
2	CK-MB	>10 U/l[1]	> 16 U/l[2]	> 25 U/l*
3	a CK-MB activity between 6 and 25% of the total CK activity			

*Calculated values

MI could be suspected, but the values obtained may be below the specified limits. To confirm if a recent infarct has occurred, the test should be repeated after 4 hours.

It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

Physiological changes in serum or plasma analyte concentrations can be caused by a number of substances. Comprehensive discussion of possible interfering substances, their serum or plasma concentrations, and their possible physiological involvements is beyond the scope of this document. The listed reference contains specific details on known potential interfering substances [5]. The user must remain vigilant to the possible effect on results of unknown interferences from medications or endogenous substances. All patient results must be evaluated in light of the total clinical status of the patient.

SPECIFIC PERFORMANCE CHARACTERISTICS

The following performance data were obtained using a RX **monza** analyser.

LINEARITY

Antibody inhibits up to 2000 U/l of CK-MM.
This assay is linear up to 2044 U/l CKMB activity.

SENSITIVITY

The minimum detectable activity of CKMB with an acceptable level of precision was determined as 23.5 U/l.

PRECISION

Within run Precision

	Level 1	Level 2
Mean (U/l)	136	161
SD	4.89	7.03
CV(%)	3.61	4.37
n	20	20

Total Precision

	Level 1	Level 2
Mean (U/l)	136	161
SD	6.83	6.56
CV(%)	5.04	4.08
n	20	20

CORRELATION

This method (Y) was compared with another commercially available method (X) and the following linear regression equation obtained:

Y = 1.0756 X - 18.799
and a correlation coefficient of r = 0.9903

43 patient samples were analysed spanning the range 34.4 to 1733 U/l.

REFERENCES

1. Stein, W., Med. Weit 1985; **36**: 572.
2. Szasz, G., and E. W. Busch. Paper presented at 3rd Eur. Congr. Clin. Chem., Brighton/England, June 1979; 3 - 8 (abstract).
3. Wurburg, U., *et al*., Clin. Chem. 1976; **54**: 357.
4. Jacobs *et al*, The Laboratory Test Handbook, 2nd edition.
5. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Washington, DC: AACC Press; 2000.

The presence of a vertical bar in the margin indicates a technical update from the previous revision.

EC REP Randox Teoranta, Meenmore, Dungloe, Donegal, F94 TV06, Ireland

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