MEDICA

IVD CE

REF 10226-4 4 x 28 mL / 7 mL

CREATINE KINASE-MB (CKMB) REAGENT
Each wedge contains usable volumes of 28 mL of R1 reagent and 7 mL of R2 reagent.

INTENDED USE
The EasyRA CKMB reagent is intended for the quantitative determination of the enzyme Creatine Kinase-MB (CKMB) in human serum and plasma using the MEDICA "EasyRA® Chemistry Analyzer" in clinical laboratories. Measurements of CK-MB activity are used in the diagnosis and treatment of myocardial infarction and muscle diseases such as progressive, Duchenne-type muscular dystrophy. For *in vitro* diagnostic use only. For professional use only.

SUMMARY AND EXPLANATION
Creatine Kinase (CK) are dimeric molecules composed of M and B subunits and exist as the iso-enzymes MM, MB, and BB.[1] The subunits M and B are immunologically distinct. CK-MM and CK-MB are distributed primarily in the skeletal muscle and heart muscle, respectively, while CK-BB is present mainly in the brain and in tissues composed of smooth muscle.[2] The determination of CK-MB activity in serum is an important element in the diagnosis of myocardial ischemia, e.g. in acute myocardial infarction, myocarditis, etc.[3,4] CK-MB is detectable in the blood about 3-8 hours after the onset of cardiac symptoms and can remain detectable over a lengthy period of time, depending on the course of the condition.[3]
Elevated CK-MB is not specific for MI (myocardial infarction) and may be detected in other disease states. Elevated CK-MB values should be interpreted in conjunction with clinical presentation and medical history.

PRINCIPLE OF THE PROCEDURE
In this procedure CK activity is measured in the presence of an antibody to CK-M monomer. This antibody inhibits the M subunits of CK-MM and CK-MB, and thus allows determination of the B subunit of CK-MB (assuming the absence of CK-BB). CK-B catalytic concentration, which corresponds to half of CK-MB concentration, is determined from the rate of NADPH formation, measured at 340nm, using the following series of reactions:

$$\text{Creatine Phosphate + ADP} \xrightarrow{\text{Creatine Kinase}} \text{Creatine + ATP}$$

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

$$\text{Glucose-6-P + NADP}^{+} \xrightarrow{\text{G6PDH}} \text{Gluconate-6-phosphate + NADPH + H}^{+}$$

The CK-MB activity is obtained by multiplying the CK-B activity by two.

REAGENTS

CK-MB Buffer Reagent (R1):

Imidazole Buffer (pH 6.3)	50 mmol/L
Glucose	20 mmol/L
N-Acetyl-L-Cysteine	20 mmol/L
Magnesium Acetate	10.0 mmol/L
NADP	2.0 mmol/L
EDTA	2.0 mmol/L
Hexokinase	5.0 kU/L
LDH	1.5 kU/L
Sodium Azide	<0.1%
Monoclonal antibodies (mouse) against human CK-M, inhibiting capacity	

CK-MB Substrate Reagent (R2):

Imidazole Buffer (pH 9.0)	50 mmol/L
Creatine phosphate	150 mmol/L
ADP	10 mmol/L
AMP	20 mmol/L
Diadenosine pentaphosphate	50 µmol/L
Glucose-6-PDH	20 kU/L
Sodium Azide	<0.1%

PRECAUTIONS

1 Good laboratory safety practices should be followed when handling any laboratory reagent. (*CLSI,* GP17-A2).
2 This material contains Sodium Azide as preservative. 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 your eyes or if ingested, seek immediate medical attention. Refer to the Safety Data Sheet for risk, hazard and safety information.
3 As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4 Do not use washed cuvettes.

INSTRUCTIONS FOR REAGENT HANDLING, STORAGE AND STABLITY

The reagent is ready to use as supplied. Unopened reagent is stable until the expiration date listed on the label if stored at 2 – 8°C. The reagent is stable on-board in the refrigerated reagent area of the EasyRA Analyzer for the number of days programmed on the RFID chip on the reagent wedge. Do not use the reagent if it is turbid or cloudy or if it fails to recover known serum control values.

SPECIMEN COLLECTION AND STORAGE/STABILITY

Clear unhemolyzed serum/plasma should be used. Plasma may be used from blood collected with lithium heparin as anticoagulant. Centrifuge and remove the serum/plasma as soon as possible after collection. Serum/plasma CK-MB is stable for 3 days at 2 – 8°C.

PROCEDURE

Materials Provided

Medica CKMB Reagent Wedge, REF 10226

Additional materials required

Commercially Available Control Material
Medica Precision Test Dye Wedge, REF 10764
Medica Cleaner Wedge – Chemistry & ISE, REF 10660 *or*
Medica Cleaner Wedge – Chemistry, REF 10661

Instructions for Use

The reagent is ready to use as supplied. Remove the cap and place the reagent in the EasyRA Analyzer reagent tray located in the reagent area. The on-board stability (30 days maximum) is programmed on the RFID chip on the reagent wedge.

Note: Check the inside of the necks of the wedge for foam after removing the caps and placing the wedge on the analyzer. If there is foam, remove it with a swab or a disposable pipette before performing the test. Use separate swabs or disposable pipettes for R1 and R2.

Calibration

Not applicable.

Quality Control

It is recommended that two levels of human serum based controls (normal and abnormal) be run with the assay daily, whenever patient samples are performed and with each reagent lot change. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction, or procedural errors. The laboratory should also follow local, state and federal quality control guidelines when using quality control materials.

Results
After completion of the assay, the Medica EasyRA Chemistry Analyzer calculates the CK-MB concentration from change in absorbance per minute, the sample volume, total reaction volume, the path length of 0.6 cm and the molar absorptivity of 6.22.

$$\text{CK-MB (U/L)} = \text{CK-B (U/L)} \times 2 = (\Delta A/\text{Min}) \times \frac{(\text{Total Volume (µl)} \times 1000) \times 2}{(\text{Molar absorptivity} \times \text{Pathlength(cm)} \times \text{Sample Volume(µl)})}$$

A unit per liter (U/L) of CK-B activity is the amount of enzyme, which oxidizes one µmol/L of NADP per minute.

Expected Values[6]
The reference range for CK-MB activity on the EasyRA Analyzer is below 24 U/L.[7,8] This range has been validated by Medica according to CLSI C28-A3c. It is recommended that each laboratory establish its own range of expected values, since differences exist among instruments, laboratories and local populations.

Procedural Limitations (e.g. if sample is above assay range)

Only unhemolyzed serum or plasma samples should be used.
If the Absorbance Change per Minute (ΔA/Min) is greater than 0.003, which corresponds approximately to 100 U/L, results will be flagged with "SD" (substrate depletion) by the analyzer. Absorbance changes per minute above this value are above the linear range of the test. If the "Re-run" icon is selected by the operator, the sample may be retested using one-fifth (1/5) the sample volume. The retest results are calculated to reflect the use of the smaller sample volume. This will extend the reportable range of the CK-MB test to 500 U/L.

Performance characteristics[9]

Reportable Range
The reportable range is 5 to 100 U/L. Extended range is 5 to 500 U/L when one-fifth of the sample is used (1:4 dilution).

Inaccuracy / Correlation (CLSI, EP9-A2)
The following table lists the data obtained in a comparison of the Medica Reagent for CK-MB (Y) on the EasyRA Analyzer to the performance of a FDA cleared CK-MB reagent (X) on the Stanbio Sirrus® Analyzer*. The data shown below represents single determinations of serum obtained on the EasyRA Analyzer vs. the average of two replicate values obtained on an FDA cleared device.

SERUM	Number of samples	41	Range of Samples	6 to 100 U/L
	Slope	0.9869	y Intercept	0.4702
	Correlation Coefficient	0.9961	Regression Equation	0.9869*X + 0.4702

** Sirrus is a registered trademark of Stanbio Laboratory LLP of 1261 North Main St. Boerne, TX 78006*

The data shown below represent single determinations for plasma (Li heparin) vs. the average of two replicate serum values obtained on the EasyRA Analyzer. All testing parameters are identical.

PLASMA	Number of samples	57	Range of Samples	5 to 96 U/L
	Slope	0.9797	y Intercept	1.4523
	Correlation Coefficient	0.9945	Regression Equation	0.9797*X + 1.4523

Imprecision (CLSI, EP5-A2)
Duplicate measurements of each of three levels of QC material were tested twice a day for 20 days. Both within-run and total precision were determined from these data.

Within run imprecision:			**Total Imprecision:**		
QC Level	**Within Run SD**	**Within Run CV**	**QC Level**	**Total Imprecision SD**	**Total Imprecision CV**
U/L	**U/L**	**%**	**U/L**	**U/L**	**%**
94	0.9	0.9	94	1.7	1.8
30	0.7	2.4	30	0.9	3.0
14	0.6	3.9	14	0.8	5.5

Linearity (CLSI, EP6-A)

Linear from 5 to 100 U/L, based on the linear regression Y = 1.0009*X – 0.2291

Limit of Blank (LOB):	1.7 U/L	(CLSI, EP17-A)
Limit of Detection (LOD):	2.9 U/L	(CLSI, EP17-A)
Limit of Quantitation (LOQ):	5.0 U/L	(CLSI, EP17-A)

Interfering Substances (CLSI, EP7-A)

Less than 10% interference was classified as “no significant interference.”
Hemoglobin interferes, even in minimal concentrations. Do no use hemolyzed samples.
No significant interference was found with levels up to 25 mg/dL of bilirubin.
No significant interference was found with levels up to 775 mg/dL of triglycerides (using Intralipid*).
No significant interference was found with levels up to 30 mg/dL of ascorbic acid.

**Intralipid is a registered trademark of Pharmacia AB, Clayton NC.*

Young provides a list of drugs and other substances that interfere with clinical chemistry tests.[10,11]

Note: The procedure may overestimate CK-MB activity values if CK-BB isoenzyme activity in the serum is high. CK-BB activity is usually absent in sera from normal individuals and patients with myocardial infarction.[7] The presence of a macro form of CK-BB in the specimen should be suspected if the CK-MB activity measured by this procedure represents more than 20% of the total CK activity.

REFERENCES

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EasyRA Assay Parameters (CKMB)

Primary Wavelength (nm)	340
Secondary Wavelength (nm)	405
Reaction Type	Enzyme (0)
Reaction Direction	Increase
Reagent Blank	No
Sample Blank	No
Max. first Interval Abs. change	0.003
Reaction Time	9.3 min
Calibration interval (maximum)	N/A
Reagent on-board stability	30 days

Serum/Plasma	
Sample volume (µl)	8.0
Diluent volume (µl)	20
Reagent volume R1 (µl)	160
Reagent volume R2 (µl)	40
Decimal Places (default values)	0
Units (default values)	U/L
Dilution Factor	1:4 (1/5 of sample used) (to extend measuring range)
Linearity	5 to 100 U/L
Molar Absorptivity	6.22

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