TiSENC

Creatine Kinase-MB (CLIA) PACKAGE INSERT

IVD

INTENDED USE

Creatine Kinase-MB (CLIA) is a chemiluminescence immunoassay test for the quantitative measurement of circulating Creatine Kinase MB (CK-MB) in human serum, plasma and whole blood, which is intended as an aid to diagnosis of myocardial tissue injury and myocardial infarction.

The assay can be used for samples over the range of 0.1 ng/mL~300 ng/mL.

The test must be performed on the ACCRE series analyzers (including ACCRE 6, ACCRE 8, ACCRE90, ACCRE100, ACCRE120 and other ACCRE series analyzers).

For *in vitro* diagnostic use only. For professional use only.

SUMMARY

Creatine Kinase (CK) mainly distributes in cytoplasm and mitochondria consisting of subunit M and B, which M and B respectively stand for muscular and brain. The molecular weight of each subunit is 40kD. Subunit M represents muscle type, subunit B represents brain type[1,2,3]. There are 4 isozymes of creatine kinase, which are CK-MM, CK-BB, CK-MB and mitochondrial creatine kinase. CK-MB mainly exists in cardiac tissue and its activity accounts for 20% of total. The molecular weight of this protein is 80kD.

The CK-MB content in serum is of great significance to diagnose acute myocardial infarction, myocardial ischemic diseases such as myocarditis. CK-MB can appear in the early days of myocardial injury, rising from 2 to 6 hours, peaking from 12 to 24 hours and returning to normal level from 36 to 72 hours. This process accompanies with ECG and chest pain, therefore serum CK-MB concentration can be utilized for assessing onset of myocardial injury, infarction volume and reperfusion injury. Moreover, as an important assistant means of diagnosing early acute myocardial infarction (AMI) and myocardial injury, the result of detecting serum CK-MB has a relative high specificity and sensitivity to myocardial tissue damage and myocardial infarction, and has a high diagnostic compliance rates[4,5,6,7].

In clinical practice, as a biomarker of AMI, CK-MB is usually used with myoglobin (MYO) and cardiac troponin I (cTnI). Serum CK-MB and MYO peak in the early phase of AMI, however, cTnI is more specific so combination of these three biomarkers for diagnosing AMI can enhance the specificity and sensitivity.

ASSAY PRINCIPLES

The principle of Creatine Kinase-MB (CLIA) hereinafter also referred to as the "CK-MB" combines a two-site sandwich immunoassay method with enzyme catalytic chemiluminescence detection (CLIA). The principle is described as following:
Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent cartridge. All of the assay steps are performed automatically by the ACCRE analyzer.

The sample is manually pipette into the sample well of reagent cartridge, then sample, CK-MB antibody labeled with alkaline phosphatase and CK-MB antibody coated magnetic particles are mixed thoroughly together, after incubating. The antigen in the sample and the antibody on the magnetic beads and the alkaline phosphatase labeled antibody form an antibody-antigen sandwich immunocomplex.

After the reaction is completed, the magnetic field adsorbes the beads, then removed unbound substance by three washings in the sample. The substrate is added to trigger the chemiluminescent reaction, the resulting chemiluminescent reaction is measured by photomultiplier tube (PMT) as relative light units (RLUs). The concentration of the substance to be detected in the sample is proportional to the intensity of the luminescence. At the end, the results are automatically calculated by the ACCRE analyzer in relation to the calibration curve stored.

CONTENT OF THE KIT

Package: 60 tests/kit with quality controls, 36 tests/kit with quality controls, 60 tests/kit without quality controls, 36 tests/kit without quality controls. The calibration kit has been traced to Roche fully automated chemiluminescence immunoassay analyzer cobas e411 and its CK-MB assay.

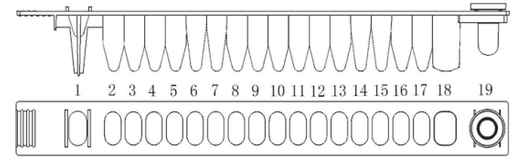
For specific calibrator concentration values, see the Calibrator Barcode Sheet for each lot.

Contents	Quantities				Notice
	REF W124A	REF W124C	REF W11A	REF W11C	
CK-MB Reagents Cartridges	60 strips	36 strips	60 strips	36 strips	Ready to Use
CK-MB Calibrator 1 (CAL1)	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Lyophilized powder,Tris buffer, 50mmol/L; ProClin300,0.48g/L; BND, 0.2g/L"
CK-MB Calibrator 2 (CAL2)	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Lyophilized powder, Human Creatine Kinase-MB protein, 5 ng/mL
CK-MB Calibrator3 (CAL3)	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Lyophilized powder, Human Creatine Kinase-MB protein, 50 ng/mL
CK-MB Quality Control Level 1 (QC1)	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	/	/	Lyophilized powder, Human Creatine Kinase-MB protein, 5 ng/mL Only for the configuration with Quality Controls
CK-MB Quality Control Level 2(QC2)	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	/	/	Lyophilized powder, Human Creatine Kinase-MB protein, 100 ng/mL Only for the configuration with Quality Controls
Instruction for Use	1	1	1	1	N/A
Reagent Registration Card	1	1	1	1	N/A
Calibrator Barcode Sheet	1	1	1	1	N/A
Quality Control Sheet	1	1	/	/	Only for the configurations with Quality Controls

Note: Refer to the target value list of the controls from Quality Control Sheet within the kit.

The Reagent Cartridge

The strip consists of 19 wells. The 1st well is the sample well and the last well 19th is the reading well for chemiluminescent signal. From the 2nd to the 18th well are all covered with a label, aluminum foil seal. The label comprises a barcodes which mainly indicates the reagent assay information, reagent lot number and expiration date. All Reagents components needed for the assay are ready-to-use and pre-dispensed in the cartridge. And please do not interchange integral component from different reagents or lots.



Description of the reagent cartridge

Well	Component	Content	Volume
3	Sample treatment solution (R3)	Tris buffer, 50 mmol/L; ProClin 300, 0.48 g/L; BND, 0.2 g/L	50 µL
5	Enzyme Label (R2)	ALP (alkaline phosphatase) labeled anti-CK-MB antibody (mouse), 2.0 mg/L; MES Buffer, 50 mmol/L; ProClin300, 0.48 g/L; BND, 0.2 g/L	50 µL
7	Magnetic Particles (R1)	Anti- CK-MB antibody (mouse) coated magnetic particles, 0.2 g/L; Tris buffer, 50 mmol/L; ProClin 300, 0.48 g/L; BND, 0.2 g/L	50 µL
10, 11, 12, 14	Wash Solutions	Tris buffer, 50 mmol/L; ProClin 300, 0.48 g/L; BND, 0.2 g/L	350 µL
16	Substrate Solution	Disodium [(4-chlorophenyl)sulfanyl] (10-methyl-9(10H)-acridinylidene) methyl phosphate	200 µL

Note: Well 1, 2, 4, 6, 8, 9, 13, 15, 17, 18 are empty.

MATERIALS AND DISPOSABLES REQUIRED BUT NOT PROVIDED

- Sample diluent (manufactured by Tisenc)
- Pipette with disposable tip to dispense 200 µL.
- Powderless and disposable gloves.
- For other specific materials and disposables, please refer to the Instrument User Manual.
- Instruments of the ACCRE series analyzers.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- For professional use only by qualified laboratory personnel.
- The kit contains products of human origin. No known analysis method can totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (see Laboratory Biosafety Manual - WHO - Geneva - latest edition).
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest; do not inhale).
- Do not use reagents after the expiration date indicated on the box label.
- Do not use if the packaging of reagent cartridge is damaged or leaked.
- Do not use the reagents if the quantity is obviously insufficient.
- If any component adheres to inside wall of wells or aluminum film of the reagent cartridge, please gently switch the reagent cartridge to let the component back the wells.
- Do not reverse the reagent cartridge. Otherwise, the result will be unreliable.
- Do not mix reagents (or disposables) from different lots.
- Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.
- Use powderless gloves, as powder has been reported to cause false results for certain enzyme immunoassay tests.

STORAGE

- Store the Creatine Kinase-MB (CLIA) kits including reagent cartridges, calibrators and quality controls **upright** at 2~8°C in dark place and the kit can be valid for 18 months from manufactured date.
- After the reagent cartridge is on-board in the ACCRE analyzer, the testing should be finished within 2 hours.
- When opened and re-dissolved, the calibrators and quality controls will be stable for 45 days at -20°C and below -20°C. Calibrators and quality controls can be frozen and thawed 3 times.
- Keep away from sunlight.
- Do not freeze reagent cartridges.

SAMPLES

The sample volume for Creatine Kinase-MB (CLIA) assay is 50 µL, please note that at least 75 µL of sample has to be pipetted manually into the reagent cartridge.

Sample type and collection

Human serum or plasma/whole blood (Heparin lithium /EDTA-K_2) are recommended.
Types of tubes:
- Plastic tube with clot activator,
- Plastic tube with clot activator and separation gel,
- Plastic tube with Heparin lithium,
- Plastic tube with EDTA-K_2.

It is recommended to validate collection tubes before using them as some may contain substances which interfere with test results.
Note: Blood sampling tube results may vary from one manufacturer to another depending on the materials and additives used.
It is the responsibility of each laboratory to validate the type of sample tube used and to follow the manufacturer's recommendations for use.

Sample preparation

The current revision of the WHO/DIL/LAB/99.1 document provides recommendations for the preparation of samples.
For use of the sample tubes, follow the tube manufacturer's instructions for use.
The pre-analytical step, including the preparation of blood samples, is an essential part of medical analyses. In accordance with Good Laboratory Practice, this step is performed under the responsibility of the laboratory manager.
Insufficient clot time can result in the formation of fibrin with micro-clots that are invisible to the naked eye. The presence of fibrin, red blood cells, or suspended particles can lead to erroneous results.
Samples containing suspended fibrin particles or erythrocyte stroma should be centrifuged before testing.
If there is a lipid layer over the sample after centrifuging, please transfer the clear sample into a new sample tube without transferring the lipid layer.
Severe hemolysis samples (hemoglobin >1000mg/dL) or heat-inactivated samples are not recommended for applying on the reagents.
Preparation of frozen-stored samples: after thawing, these samples must be thoroughly mixed before testing.
Mix using with a vortex-type mixer. If necessary, clarify samples by centrifuging before testing.

Sample stability

Serum and plasma sample can be stored at 15-30°C for up to 4 hours or at 2-8°C for up to 24 hours; if longer storage is required, freeze the serum or plasma at -20 °C or below -20 °C for up to 3 months, without exceeding 1 freeze/thaw cycle.

Whole blood samples can be stored at 15-30°C for up to 4 hours or at 2-8°C for up to 8 hours and not undergo freeze/thaw cycles.

INSTRUCTIONS FOR USE

For complete instructions, see the Instrument User Manual.

When using the assay for the first time: register the assay using the registration code in the reagent kit before any assay is used in the ACCRE analyzer for the first time. Calibration, QC and sample test are only available after assay was registered successfully.

When opening a new lot of reagents. Each new lot of reagent must be registered by using the registration code in Reagent Registration Card within the reagent kit.

Calibration

Calibration must be performed with the reagents and calibrators from Manufacturer. Each bottle of lyophilized calibrator should be re-dissolved by purified water to 1.0mL before use.

Calibration is necessary under the following conditions:

(1) New reagent lot number applied;

(2) Same lot of reagents used more than 4 weeks;

(3) Quality Control out of control;

(4) When necessary, for example: system maintenance or repair that may affect the analytic performance.

Test Procedure

1. Remove the kit from storage at 2-8 °C and take out the required number of reagent cartridge. Allow the reagent cartridges, specimens, calibrators, and/or controls to equilibrate to room temperature (15-30°C) prior to testing.
2. Use each assay CK-MB strips for each sample, calibrator or control respectively.
3. Place the reagent cartridge into the reagent rack.
4. Manually pipette the Sample, Calibrator, or Controls into the sample well of each reagent cartridge.
   Please note that at least 75μL of sample has to be pipetted manually into the reagent cartridge and 50μL sample is needed for each test.
   ** Before pipetting: Mix the samples using a vortex-type mixer if necessary, and ensure that the samples and sample diluent (if applicable) DO NOT have any bubbles. The same for calibrators and controls.*
5. Put the sample rack into the reagent chamber of ACCRE analyzer.
6. Place the assay tips into the reagent chamber of ACCRE analyzer.
7. Input Sample ID, sample type, dilution etc. into the software as directed by the USER MANUAL.
8. Creatine Kinase-MB (CLIA) assay will be completed within approximately 15 minutes. Assays will be performed automatically by the ACCRE analyzer.
9. After assay is completed, remove the reagent rack from the ACCRE analyzer. Dispose the used reagent cartridge into an appropriate recipient.

Dilution

Samples with concentrations above 300 ng/mL can be diluted with sample diluent manually. The recommended dilution is 1:2. And the concentration after dilution should be more than 150 ng/mL. After manual dilution, multiply the result by the dilution factor. ACCRE analyzer cannot perform automatic dilution for samples.

Quality control

Each bottle of lyophilized quality control should be re-dissolved by purified water to 1.0mL before use.

Quality control can be performed in accordance with local regulations or requirements related to accreditation, as well as requirements defined in the laboratory's quality control procedure.

RESULTS AND INTERPRETATION

Calculation of Results

The results are automatically calculated by the ACCRE analyzer using stored calibration curve which generate by 3-point calibration, and calculated according to a predefined mathematical model, the results expressed in ng/mL.

Interpretation of Results

The detection range is from 0.1 ng / mL to 300 ng / mL. For samples with CK-MB below the upper limit of detection, quantitative determination can be performed. if the sample is lower than the lower limit of detection, the reported result is <0.1ng/mL; if the sample is higher than the upper limit of detection, the reported result is >300ng/mL.

Results of Creatine Kinase-MB (CLIA) assays are determined by testing serum specimens from a study in clinical centers with group of totally 321 healthy adult individuals in China (171 male and 150 female). The 97.5th and 99th percentile values of male are respectively 5.010 ng/mL and 6.362 ng/mL. The 97.5th and 99th percentile values of female are 3.714 ng/mL and 4.971 ng/mL, respectively.

** Please note that results may differ between laboratories due to variations in population and test method. If necessary, each laboratory should establish its own reference range.*

In interpreting the results, patient's clinical condition including symptoms, diseases history, and other relevant data and information should be considered.

LIMITATIONS

1. If the test results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
2. For diagnostic purposes, results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.
3. Please note that heterophile antibody in human serum or plasma can interfere the test result.
4. High concentration of HAMA can also cause a false positive or false negative result.

PERFORMANCE CHARACTERISTICS

Detection limits

LOB (Limit of Blank) = 0.1 ng/mL.

LOB was determined according to CLSI ® EP17-A2 recommendations

Measurement range

The measurement range is the range of values corresponding to the acceptable performance limits (precision and linearity).

The measurement range of the Creatine Kinase-MB (CLIA) assay is: 0.1 ng/mL ~ 300 ng/mL.

Linearity

Linearity was evaluated according to CLSI ® EP06-A recommendations. The Creatine Kinase-MB (CLIA) assay is linear between 0.3 ng/mL to 300ng/mL, The absolute linear correlation coefficient (r) should be bigger than 0.9900.

Precision

A precision study was performed according to CLSI ® EP05-A3 recommendations.

The within-lot precision CV% is less than 8%.

The between-lot precision CV% is less than 10%.

Accuracy

The sample which is a control with trueness traceability is used for the detection, and the relative deviation between the detection result and the calibration concentration is within ±10%.

Cross-reactivity

The Creatine Kinase-MB (CLIA) assay is specific for Creatine Kinase MB. The following proteins were tested and found to have an insignificant effect on the measured CK-MB.

Substance	Concentration	Cross-reactivity rate
CK-BB	150 ng/mL	≤0.1%
CK-MM	300 ng/mL	≤0.1%

Interfering Substances

Potential interference substances were studied according to CLSI ® EP07-A3 recommendations. No significant interference was detected up to the maximum concentrations tested.

Substance	Concentration
Hemoglobin	≤1000 mg/dL
Bilirubin	≤34 mg/dL
Triglyceride	≤1500 mg/dL
Total Protein	≤5 g/dL
Rheumatoid factor	≤1500 IU/mL

HAMA

Patient samples containing human anti-mouse antibodies (HAMA) may give falsely elevated or decreased values. Although HAMA-neutralizing agents are added, extremely high HAMA serum concentrations may occasionally influence results[8-11].

Hook effect

No hook effect was found up to Creatine Kinase MB (CK-MB) concentrations of 5000 ng/mL.

WASTE DISPOSAL

Dispose used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.

It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

INDEX OF SYMBOLS

Symbol	Meaning	Symbol	Meaning	Symbol	Meaning
	Consult instructions for use		Contains sufficient for <n> tests		Date of manufacture
	*In vitro* diagnostic medical device		Use-by date		Do not re-use
	Temperature limit		Batch code		Catalogue number
	Keep away from sunlight		Keep dry		Manufacturer
	Authorized representative in the European Community/ European Union		Do not use if package is damaged and consult instructions for use		Contains biological material of animal origin
	Biological risks		Caution		CE marking
	Upright		Fragile, handle with care		Do not roll
	Control				

REFERENCES

1. Van der Veen KJ, Willebrands AF. Isoenzymes of creatine phosphokinase in tissue extracts and in normal and pathological sera. Clin Chim Acta 1966; 13: 312.
2. Neumeier D. Tissue specific and subcellular distribution of creatine kinase isoenzymes. In: Lang H, editor. Creatine Kinase Isoenzymes. New York: Springer-Verlag, 1981: 85-109.
3. E Jockers-Wretou, G Pfleiderer. Quantitation of creatine kinase isoenzymes in human tissues and sera by an immunological method. Clin Chim Acta 1975; 58: 223-32..
4. Galen RS, Gambino R. Isoenzymes of CPK and LDH in myocardial infarction and certain other diseases. Pathobiol Annu 1975; 5: 283-315.
5. Galen RS. Isoenzymes and myocardial infarction. Diagn Med 1978; 1: 40-52.
6. Lott JA, Stang JM. Serum Enzymes and isoenzymes in the diagnosis of myocardial ischemia and necrosis(Review). Clin Chem 1980; 26: 1241-50.
7. The Joint European Society of Cardiology/American College of Cardiology Committee. Myocardial infarction redefined—a concensus document of the Joint European Society of Cardiology/American College of Cardiology committee for the redefinition of myocardial infarction. J Am Coll Cardiol 2000; 36: 959-69.
8. Boscato Lm, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988;34: 27-33.
9. Kricka L. Interferences in immunoassays - still a threat. Clin Chem 2000; 46: 1037-1038.
10. Bjerner J, et al. Immunometric assay interference: incidence and prevention. Clin Chem 2002; 48: 613-621.
11. HHS Publication, 5th ed., December 2009. Biosafety in Microbiological and Biomedical Laboratories.

Shenzhen Tisenc Medical Devices Co., Ltd.
Address: 11F, 11G, Kechuang Building, Quanzhi Science and Innovation Park, Maozhoushan Industrial Park, Shajing Community, BaoAn District, Shenzhen, 518104, P.R.China
Phone: +86-0755-23225620
E-mail: globalmarketing@tisenc.com

EC REP

Shanghai International Holding Corp. GmbH (Europe)

Address: Eiffestrasse 80, 20537 Hamburg, Germany
Phone: +49-40-2513175
Fax: +49-40-255726
DIMDI No.: DE/0000040627
E-mail: shholding@hotmail.com

Eng 20240201-Ver 8.0