# Instruction For Use

**【Product Name】**

Creatine Kinase MB (Electrochemiluminescence Immunoassay)

**【Order Information】**

| REF NO. | Package Size |
|---|---|
| 691039 | 50T |
| 691040 | 2×50T |
| 691017 | 100T |
| 691018 | 2×100T |

**【Intended Use】**

Immunoassay for in vitro quantitative determination of creatine kinase MB in human serum and plasma. The results can be used in assisting diagnosis of myocardial injury.

Creatine kinase is a dimeric enzyme, consisting of M and B subunit. Creatine kinase in human body occurs in four different forms: creatine kinase MB (CK-MB), CK-BB (brain type), cytosolic isoenzymes CK-MM (muscle type) and mitochondrial isoenzymes (CK-MiMi). The proportion of CK-MM is very large, the proportion of CK-MB is less than 5% of total activity and the proportion of CK-BB is minimal in healthy human serum. CK-MB is expressed in the myocardium. The level of CK-MB in the peripheral blood will increase when CK-MB is released to the peripheral blood caused by the myocardial damage[1]. CK-MB is released when myocardial tissue is seriously damaged, so CK-MB has some limitations in the diagnosis of early stage of myocardial injury. The determination of CK-MB is often combined with MYO and cTnI which can improve the specificity and sensitivity in clinical diagnosis[2-3].

**【Test Principle】**

Sandwich principle. Total duration of assay: 18 minutes.

1st incubation: 15 μL of sample, biotinylated monoclonal CK-MB-specific antibody and monoclonal CK-MB-specific antibody labeled by ruthenium trisbipyidyl NHS ester form a sandwich compound by immunoreaction.

2nd incubation: After addition of streptavidin-coated micro magnetic beads, the compounds bind to the micro beads via interaction of biotin and streptavidin.

Measurement: The reaction mixture is aspirated into the measuring cell where the micro beads and immuno-compounds are magnetically captured onto the surface of the electrode.

Unbound substances are then removed by Buffer. Application of a voltage to the electrode then induces emission of luminescence which is measured by a photomultiplier.

Results are determined via calibration and a master curve provided via the reagent barcode.

**【Main Components】**

The reagent pack consists of MB, RA, RB, calibrators. different lots cannot be used at the same time or mixed up together.

| Components | Ingredients | Volume (50T) | Volume (2×50T) | Volume (100T) | Volume (2×100T) |
|---|---|---|---|---|---|
| (MB) | Streptavidin-coated micro magnetic beads, 0.75 mg/mL: 0.1M PBS; 0.05% ProClin™ 300 | 1×3.0mL | 2×3.0 mL | 1×5.0 mL | 2×5.0 mL |
| (RB) | Anti-CK-MB-Ab~biotin, 2.5mg/L: 0.1M PBS; 0.05% ProClin™ 300 | 1×5.8mL | 2×5.8 mL | 1×9.0 mL | 2×9.0 mL |
| (RA) | Anti-CK-MB-Ab~Ru(bpy)$_3^{2+}$, 1.2 mg/L: 0.1M PBS; 0.05% ProClin™ 300 | 1×5.8mL | 2×5.8 mL | 1×9.0 mL | 2×9.0 mL |
| Calibrators | Calf serum, 0.05% ProClin™ 300; lyophilized | High: 1×1.0 mL<br>Low: 1×1.0 mL | | | |

Traceability: This method can be traced back to the Roche Elecsys CK-MB.

Materials and instruments required but not provided:

Additional materials for Automated ECL Immunoassay Analyzer eCL8000, eCL8000i, eCL8000p, eCL8000x:

- REF 679005, Auffer, 480 mL, or REF 679004, Auffer, 6×480 mL
- REF 679007, Buffer, 480 mL, or REF 679006, Buffer, 6×480 mL
- REF 679009, Concentrated Washing Buffer, 1 L, or REF 679008, Concentrated Washing Buffer, 6×1 L
- REF 679011, Assay Cup, 300 T, or REF 679012, Assay Cup, 3000 T
- REF 691027, Multi Control Cardiac Marker, Low value: 3×2.0 mL, High value: 3×2.0 mL, or REF 691028, Multi Control Cardiac Marker, Low value: 6×2.0 mL, High value: 6×2.0 mL

Additional materials for Automated ECL Immunoassay Analyzer eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p:

- REF 0320501804, Auffer, 2×1 L, or REF 0320501805, Auffer, 4×1 L
- REF 0320501904, Buffer, 2×1 L, or REF 0320501905, Buffer, 4×1 L
- REF 0320507502, PreClean, 2×800 mL, or REF 0320507503, PreClean, 4×800 mL
- REF 320510001, Assay Cup/Assay Tip, 6×6×105, or REF 032120111, Assay Cup/Assay Tip, 12×2×105
- REF 691027, Multi Control Cardiac Marker, Low value: 3×2.0 mL, High value: 3×2.0 mL, or REF 691028, Multi Control Cardiac Marker, Low value: 6×2.0 mL, High value: 6×2.0 mL

Additional materials for Automated ECL Immunoassay Analyzer eCL9000, eCL9600, eCL9900, eCL9900i:

- REF 0320501801, Auffer, 2×2 L, or REF 0320501806, Auffer, 4×2 L
- REF 0320501901, Buffer, 2×2 L, or REF 0320501906, Buffer, 4×2 L
- REF 0320507501, PreClean, 2×2 L, or REF 0320507504, PreClean, 4×2 L
- REF 0320510001, Assay Cup/Assay Tip, 6×6×105, or REF 0320510002, Assay Cup/Assay Tip, 1×6×105
- REF 691027, Multi Control Cardiac Marker, Low value: 3×2.0 mL, High value: 3×2.0 mL, or REF 691028, Multi Control Cardiac Marker, Low value: 6×2.0 mL, High value: 6×2.0 mL

**【Storage and Shelf Life】**

Store at 2°C-8°C.

Do not freeze.

Store the reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Damaged, expired or contaminated reagents should be discarded.

| *Stability of the reagents* | |
|---|---|
| Unopened at 2°C~8°C | 18 months |
| Store at 2°C~8°C after opening | 8 weeks |
| Stored on the analyzers at 2°C~15°C | 8 weeks |

The calibrators and control materials are stable up to the stated expiration date.

| *Stability of the calibrators* | |
|---|---|
| Unopened store at 2°C~8°C | 18 months |
| Store at 2°C~8°C after reconstituted | 8 hours |
| Store at -25°C~-15°C after reconstituted | 4 months (freeze only once) |

**【Applicable Instrument】**

Automated ECL Immunoassay Analyzers: eCL8000, eCL8000i, eCL8000p, eCL8000x, eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p, eCL9000, eCL9600, eCL9900, eCL9900i.

**【Specimen collection, handling and storage】**

Human serum and plasma added with heparin lithium, heparin sodium and EDTA-2K, EDTA-3K anti-coagulants are recommended. Blood samples should be collected by standard operation of venous puncture; after complete coagulation, the tangible component should be removed by centrifugation. The sample should avoid bubbling during testing. Lipid layer floating on the upper of the sample should be removed. Samples with severe hemolysis are not recommended. Samples would better to be tested in 4 hours after collection, otherwise, should be stored 2~8°C for no more than 8 hours or -25~-15°C for 90 days. Freezing and thawing cycle is permitted only once. The samples should be centrifugated before test if samples are deposited and frozen. Taking into account the possible factor of evaporation, the test of samples, calibrators and control materials is performed as short as possible within 2 hours.

**【Assay Procedure】**

**Testing procedures and precautions**

Previous to operation, one should read the operational manual carefully to obtain system operation procedure, sample processing, security precaution, maintenance and other related information.

Set up Creatine Kinase MB (CK-MB) test according to the operational manual.

Put the CK-MB rackpack into the correct analyzer slot to automatically suspend magnetic beads at least 30 min before testing.

Recommended ambient temperature:10~30°C; Relative humidity: ≤ 80%.

**Handing of lyophilized calibrators**

Carefully dissolve the contents of calibrator by adding exactly 1.0 mL of distilled or deionized water and allow to stand closed at room temperature for at least 15 minutes to reconstitute. Mix carefully, avoiding foam formation. Transfer aliquots of the reconstituted calibrators to the additional plastic bottles. After bottles is marked and dispensed. If you do not test immediately, you need to store the aliquots immediately at -15°C or lower than -15°C.

**Calibration**

Calibration should be performed using lot-matching reagent and calibrators.

Before calibration, the reagent and main curve information should be imported into the analyzer via radio frequency identification (RFID) reagent card (refer to instrument user manual). The analyzer adjusts the main curve to produce working curve according to the calibrator results, and identifies validity of the working curve automatically.

Recalibration is recommended when:

(1) When using a new lot of reagent;

(2) When the same lot of reagents has been used on the analyzer for more than 4 weeks;

(3)If the quality control result exceeds the defined limit;

(4)When changing the buffers of different lot numbers.

**Quality control**

For quality control, use Multi Control Cardiac Marker.

In addition, other suitable control material can be used.

Control materials for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Please make sure that the correct values are used..

**Calculation**

The system software automatically calculates the analyte concentration using particular algorithm, and the result unit is ng/mL or μg/L : 1 ng/mL = 1 μg/L.

**Specimen dilution**

Samples exceeding the measuring range would be manually diluted with CK-MB-negative serum.

The recommended dilution ratio is 1:2, the concentration of the diluted sample must be > 50 ng/mL or μg/L, and the diluted sample test results need to multiply dilution ratio.

**【Biological reference interval】**

According to serum test results of 398 healthy human (males 237, females 161) in Guangdong Province, P. R. China, the percentile 97.5 to 99 gives male reference range of 4.820~5.501 ng/mL and female reference range of 3.753~4.472 ng/mL.

Due to differences in the region, race, gender and age, laboratories are recommended to set up their own reference ranges.

**【Result Interpretation】**

For explaining the result, the patient's overall clinical manifestation should be referred to, including symptoms, medical history and other relevant data and information.

**【Limitations】**

Test results are used only for clinical reference and cannot be used as the basis for diagnosis or rule-out of diseases alone.

Even the concentration of CK-MB reached 10000 ng/mL, hook effect is unobservable.

When the sample containing bilirubin ≤ 400 μg/mL, triglyceride ≤ 30 mg/mL, total protein ≤ 100 mg/mL, biotin ≤ 30 ng/mL, or hemoglobin ≤ 5 mg/mL, or HAMA (human anti-murine antibodies) ≤ 320 ng/mL, interference deviation to the test result lies within ±10%. The test result would not be interfered by rheumatoid factor (3000 IU/mL).

**【Measuring Range】**

The measuring range of the kit is 0.3~280 ng/mL. For samples with CK-MB content below the lower detection limit, the results are reported in < 0.3 ng/mL; If the CK-MB content is above the upper detection limit, then the sample should be diluted and re-tested. The results are reported after multiplying with the dilution factor.

**【Analytical Performance】**

**Lower limit of measurement**

The lower detection limit is 0.3 ng/mL (repeatability study, n = 20).

**Accuracy**

Determination of CK-MB additive samples, the recovery rate should be within 90%-110%.

**Linearity**

The correlation coefficient (Pearson's r) is not less than 0.9900 in the interval of 0.3~280 ng/mL.

**Within-run precision (repeatability)**

The coefficient of variation (CV)is no more than 7.5%.

**Between-lot precision**

The coefficient of variation (CV) is no more than 10%.

**Analytical specificity**

Test results of blank samples spiked with analogs below are lower than 0.5 ng/mL.

| Analog | Concentration (ng/mL) |
|---|---|
| CK-MM | 10000 |
| CK-BB | 10000 |

**Method Comparison**

A comparison of the Lifotronic CK-MB assay (y) with the Roche Elecsys CK-MB (x) using clinical samples gave the following correlations:

Number of serum samples measured: 229

Linear regression:

y=1.0437x +1.2009

r = 0.975

The sample concentrations were between 0.341 and 240.0 ng/mL

**【Precaution and Warning】**

The kit is only used for in vitro diagnostics.

Exercise the normal precautions required for handling all laboratory reagents.

All reagents and samples including specimen, reagents, calibrators and control materials should avoid foaming before and during test.

Disposal of all waste material should be in accordance with local guidelines.

Safety data sheet available for professional user on request.

This product contains animal-sourced materials and may have potential biological risk. All samples and test wastes should be treated as the source of infection, and all wastes must be disposed according to local regulations.

Any serious incident that would be occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

All samples and test wastes should be treated as the source of infection, and all wastes must be disposed according to local regulations.

The preservative ProClin™300 which contain Mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1): May cause an allergic skin reaction. thus, proper personal protection should be adopt while handling the reagent: avoid breathing dust/fume/gas/mist/vapours/ spray, wear protective gloves and contaminated work clothing should not be allowed out of the workplace. If skin irritation or rash occurs: get medical advice/attention, take off contaminated clothing and wash it before reuse, and dispose of contents/container to an approved waste disposal plant.

**【Symbols Explanation】**

| Symbol | Title of Symbol | Symbol | Title of Symbol |
|---|---|---|---|
|  | Manufacturer |  | Consult instructions for use |
|  | Use-by date | IVD | In vitro diagnostic medical device |
| LOT | Batch code |  | Sufficient for <n> tests |
| SN | Serial number |  | Biological risks |
|  | Temperature limit |  | This way up |
| EC REP | Authorized representative in the European Community | CE | Indicates this device is in compliance with Europe Directive. |
| REF | Catalogue number | P/N | Material code |
|  | Warning |  | Date of manufacture |
| UDI | Unique device identifier | | |

**【Literature References】**

1. Panteghini M. Diagnostic application of CK-MB mass determination. Clinica Chimica Acta, 1998, 272(1): 23-31.
2. De Winter R J, Koster R W, Sturk A, et al. Value of myoglobin, troponin T, and CK-MB mass in ruling out an acute myocardial infarction in the emergency room. Circulation, 1995, 92(12): 3401-3407.
3. Kost G J, Kirk J D, Omand K. A strategy for the use of cardiac injury markers (troponin I and T, creatine kinase-MB mass and isoforms, and myoglobin) in the diagnosis of acute myocardial infarction. Archives of Pathology & Laboratory Medicine, 1998, 122(3): 245.

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