Human cardiac troponin I CLIA Kit

Chemiluminescence Immunoassay for the quantitative determination of Cardiac troponin I in human serum

REF SKT-071 CE IVD 50,100

INTENDED USE

Human cardiac troponin I CLIA Kit is a Chemiluminescence Immunoassay (CLIA) intended for the quantitative measurement of human Cardiac troponin I in serum.

For in-vitro diagnostics purposes only

SUMMARY OF PHYSIOLOGY

The kit is used for clinical in vitro quantitative detection of troponin I concentrations in human serum.

A majority of the troponin complex (94 - 97%) is localized on the (thin) actin filament in the sarcomere of high-sensitivity Cardiac and skeletal muscles.[1-2]The troponin complex consists of three different proteins: troponin I, troponin C, and troponin T. Three tissue-specifics troponin I subunits have been identified, each coded by different genes: Two of these subunits are fast/slow isoforms of troponin I (sTnI) derived from the fast-twitch/ slow - twitch fibres of skeletal muscles. The third is the myocardial forms known as Cardiac troponin I (cTn I) whose primary structure differs considerably from that of the two skeletal muscle isoforms.[4-8]

Troponin I is a specific and sensitive marker for the detection of myocardial damage. As early as 4 to 12 hours after acute high-sensitivity Cardiacischaemia, elevated troponin I levels (above the specific cut-off) allow diagnosing acute myocardial infarction with high specificity and sensitivity. According to the Joint Consensus of the ESC and ACC–Myocardial infarction redefined-for the definition of myocardial infarction and the IFCC C-SMCD quality specifications for cTn I assays, AMI should be defined as any Cardiac Troponin I concentration exceeding the 99th percentile of a reference control group. Accordingly, the acceptable imprecision (total) at the 99thpercentile for each assay should be defined as not exceeding10%.[9-10]

Peak troponin I concentrations are reached after 14 to 36 hours and remain at a high level for up to 7 days after the acute event. Serial testing of troponin I concentrations is recommended in patients with suspected myocardial damage. According to their commendation of the IFCC Committee for the use of high-sensitivity Cardiac markers in coronary artery diseases, troponin I should be determined at admission as well as 4, 8 and 12 hours (or in the next morning) after hospitalization. Ample data suggests that patients with unstable angina pectoris (UAP), in whom troponin levels are concomitantly elevated (above the reference range) have a significantly increased risk of mortality due to cardiovascular disease. Therefore, troponin I is a suitable parameter for risk stratification of such patients. [11]

Successful risk stratification requires, however, analytical methods sensitive enough to detect even minor elevations of troponin I(above the reference range) early and with high precision.

ASSAY PRINCIPLE

The Human cardiac troponin I CLIA Kit is designed, developed, and produced for the quantitative measurement of human cTn I level in serum samples. The assay utilizes a two-site "sandwich" technique with two antibodies that bind to different epitopes of cTn I.

Assay calibrators, controls, or patient serum samples are added directly to a reaction vessel together with magnetic particles antibody. The magnetic particles capture the cTn I in the form of "magnetic particles–cTn I antibody–cTn I–acridinium ester cTn I antibody". Materials bound to the solid beads are held in a magnetic field while unbound materials are washed away. Then trigger solutions are added to the reaction vessel, and light emission is measured with the ECL100 or ECL 25 analyzer. The relative light units (RLU) are *proportional* to the concentration of a PRL in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve and reported in serum cTn I concentration.

REAGENTS: PREPARATION AND STORAGE

This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date. It can be stored for 1 month at 2 – 8°C after kit opening.

1. cTn I Magnetic Particle Solution (07101)

Qty:	3.5mL (50/kit),6.0mL (100/kit)
Storage:	2 – 8°C
Preparation:	Ready to Use

2. Acridinium ester cTn I antibody (07103)

Qty:	3.5mL (50/kit),6.0mL (100/kit)
Storage:	2 – 8°C
Preparation:	Ready to Use

3.cTn I Calibrators (07107-07108)

Qty:	2 x vials
Storage:	2–8°C before reconstitution, <20°C after reconstitution; Do not exceed 6 freeze-thaw cycles
Preparation:	Must be reconstituted with 0.5 mL of demineralized water and then mixed by inversions or gentle vortexing. Make sure that all solids are dissolved completely and there are no air bubbles prior to use.

4.cTn I Controls (07109-07110)

Qty:	2 x vials
Storage:	2–8°C before reconstitution, <20°C after reconstitution; Do not exceed 6 freeze-thaw cycles
Preparation:	Must be reconstituted with 0.5 mL of demineralized water and then mixed by inversions or gentle vortexing. Make sure that all solids are dissolved completely and there are no air bubbles prior to use.

SAFETY PRECAUTIONS

The reagents must be used in a professional laboratory environment and are for in vitro diagnostic use. Source material which contains reagents of bovine serum albumin was derived New Zealand. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they were potentially infectious. Avoid contact with reagents containing hydrogen peroxide. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Exercise Good Laboratory Practices.

MATERIALS REQUIRED BUT NOT PROVIDED

1. ECL100 Immunoassay Analyzer (ECL100) or ECL25 Immunoassay Analyzer (ECL25)
2. CL011 Cuvettes (for ECL100) or CL010 Cuvettes (for ECL25)
3. EDI™ Wash Reagent (P-594)
4. EDI™ Trigger Solutions A and B (P-595)

The instrument must operate using materials supplied by Epitope Biotechnology, Co.,Ltd. or Epitope Diagnostics, Inc. When materials are sourced from third-party suppliers are being used, Epitope Biotechnology,Co.,Ltd. and Epitope Diagnostics, Inc. takes no responsibility of the validity for obtained results. Materials are available to purchase from Epitope Biotechnology,Co.,Ltd. and Epitope Diagnostics, Inc. Please contact your distributor for more information.

SPECIMEN COLLECTION AND PREPARATION

1. Blood sample should be collected under sterile conditions.
2. For human serum samples, other body fluids and samples may not get accurate results.
3. Clinical samples shall be tested within 2 hours after collection; If the measurement cannot be completed within 2 hours, please store the following way:
    - storage at low temperature and away from light (2℃~8℃) for 7 days
    - storage at -20℃ or below for 30 days
    - Freeze and thaw only once
4. Avoid heating inactivated samples, mixed, contaminated and hemolysis samples should be discarded.
5. Samples should be restored to room temperature before testing. Samples stored in freezer should be completely melted, and mixed evenly before use. Due to possible volatilization, samples, calibrators, and controls on the ECL platform should be tested within 2 hours.
6. Some substances in the samples will interfere with the test results. The common interfering substances and maximum allowable concentrations are bilirubin: 50 mg/dL, triglyceride: 2000 mg/dL, hemoglobin: 500 mg/dL,
7. A single assay of this item requires 50 µL sample. This quantity does not include the amount of dead volume in the sample container or the capacity required for retesting and other measurement items. For the definition of minimum required sample size, refer to the equipment manual.

CALIBRATION

An active calibration curve is required for all tests. Calibration is required for the first-time use of a reagent lot and is valid for 28 days. However, we recommend calibration every 14 days after initial calibration or when either kit control is out of range. Refer to appropriate system manuals for configuring calibrators.

QUALITY CONTROL

The characteristics of patient samples are simulated through controls and are critical to validate the performance of CLIA assays due to the random-access format. Use of controls is left to the discretion of the user, based on good laboratory practices, requirements, and applicable laws. Quality control results that do not fall within acceptable ranges may indicate invalid test results.

1. Reagents from different kit lot numbers should not be combined or interchanged. Make sure that there are no air bubbles in any reagents, calibrator, and control vials.

2. Reagent Preparation

2.1 Remove reagent cartridges from packaging and replace the solid caps with the provided soft caps for ECL100. For ECL25, carefully remove the aluminum foil seal on each container on the cartridges.

2.2 For the ECL100, take out the Magnetic Particle bottle make sure to roll between hands and gently but thoroughly mix until the magnetic particle solution is homogenous. The solution should be uniform with no clumps of magnetic particles visible; this step is vital for assay performance.

- Note: For ECL 100, if the Magnetic Particle Solution volume is over 3 mL, it will be provided in a glass bottle. It will need to be transferred from the glass bottle to the plastic vial in the cartridge with the rest of the reagents. Make sure the Magnetic Particle Solution is mixed well before transferring.

2.3 For ECL25, mix the magnetic beads by moving back and forth the bottom part of the cartridge at upright position. Make sure to look inside the cartridge until the solution is uniform with no clumps of magnetic particles visible and no air bubbles. Recap the bottle. Open the top soft cap of all reagent bottles, leaving only the hollow soft rubber.

2.4 The reagents are now ready to be loaded into the ECL100 or ECL 25 for calibration.

3. Assay Program

The following table illustrates the protocol used by the ECL100 or ECL25 for instrument operation.

Component	Quality Control Hole (µL)	Sample Hole (µL)
cTn I Controls (07109-07110)	50	-
Samples	-	50
cTn I Magnetic Particle Solution (07101)	50	50
Acridinium ester cTn I antibody (07103)	50	50
Incubate at 37°C for10 minutes		
Wash the reaction cuvette 3 times with wash reagent.		
Trigger Solution A (P-595)	100-200	100-200
Trigger Solution B (P-595)	100-200	100-200

NOTE FOR ASSAY PROCEDURE

All the reagents in this kit are ready-to-use. Make sure that there are **no air bubbles** in any reagents, calibrator, and control vials. Reagents from different kit lot numbers must not be combined or interchanged.

Please read the reagent instructions and equipment instructions carefully before using this kit and perform the test according to relevant requirements. When reagents are loaded, the

equipment will automatically stir the magnetic particles to resuspend them. Allow the reagent to mix for minimum 15 min before starting the assay program.

INTERPRETATION OF RESULTS

1. The default unit for cTn I project is pg/mL

2. Due to methodological differences or antibody specificity, there may be deviations between the test results of reagents from different manufacturers, so direct comparisons should not be made to avoid false interpretation.

3. When the concentration of cTn I in the sample exceeds 50.0 ng/mL, the sample can be diluted is recommended before detection.

4. When the sample concentration of Cardiac troponin I is lower than the detection lower limit, the test result can be reported as <10pg/mL. When the sample concentration is higher than the detection upper limit, it can be reported as >50000pg/mL.

EXPECTED VALUES

Normal reference value<254.07pg/mL.

Each laboratory should evaluate the applicability of this reference range through experiments and establish their own reference rangeif necessary.

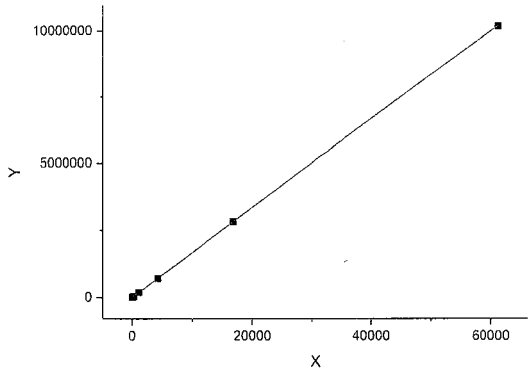
LIMITATIONS OF THE PROCEDURE

1. This product is for use on the ECL100 Immunoassay Analyzer or ECL 25 Immunoassay Analyzer only. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, operation, system performance, instructions, calibration, precautions, hazards, maintenance, and troubleshooting.
2. Reagents from different kit lot numbers should not be combined or interchanged.
3. Test results obtained from the proposed kit should not be served as a sole basis for clinical diagnosis or patient management.
4. If the test sample result is higher than the upper limit of the calibration curve, it is recommended to re-measure after dilution according to a certain ratio. The measured value is recalculated according to the dilution ratio to ensure the accuracy of the result.

PERFORMANCE CHARACTERISTICS

1. **Example of Calibration curve**

   The calibration curve is built in the calibration card. This curve is lot dependent. Here is an example of the 7-point calibration curve.



2. **Hook Effect:**
   - The assay showed no hook effect up to 1000 ng/mL.

3. **Limit of Detection (LoD):**

The limit of detection (LoD) was determined using fivesamples in the replicate of twenty. The LoD was found to be10pg/mL

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4. **Linearity:**
   - 10pg/mL to 50000pg/mL
   - Linearity correlation coefficient R ≥0.990.
5. **Accuracy**

Accuracy was determined in a triplicate using two standards and two samples. The obtained average concentration value should meet ±10% of original concentration values. The results are as follows.

Sample	Average Concentration (pg/mL)	Target Range (pg/mL).	Result
Std 1	272.9	254.403-310.937	Pass
Std 2	4025.43	3815.622-4663.538	Pass
sample 1	40.1	36.09-44.11	Pass
sample 2	330.44	290.7-355.3	Pass

6. **Intra-assay repeatability**

The intra-assay precision was determined by testing eight replicates of two samples and one control

Sample	Average Concentration (pg/mL)	SD	CV (%)
Sample1	85.6	0.1	1%
Sample2	1547.2	0.3	4%
Ctrl 1	822.6	0.4	2%

7. **Inter-assay reproducibility**

The inter-assay reproducibility was determined by testing ten replicates of two controls. The results are summarized below:

Control	Average Concentration(pg/mL)	SD	CV (%)
1	79.71	0.12	0.8%
2	1096.87	0.4	3.4%

NOTES

1. Read the instructions carefully and gently mix the reagent well before use. Avoid any air bubble before loading the reagents onto the equipment.
2. Keep the reagent in storage condition as indicated in this IFU and on the reagent label. Do not freeze reagents.

3. Avoid contact with skin, eyes and mucous membrane, and flush the contact area with clean water immediately.
4. All patient samples must be treated as potential infectious material.
5. Components in different kits cannot be mixed.
6. All waste must be disposed complying with local regulations and laws

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Biotechnology Co,Ltd and its distributors DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Biotechnology Co,Ltd.. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

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TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

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GLOSSARY OF SYMBOLS (EN 980/ISO 15223)