Lifotronic

CE

Instruction for Use

【Product Name】

Troponin I (Electrochemiluminescence Immunoassay)

【Order Information】

REF NO.	Package Size
691027	50T
691028	2×50T
691011	100T
691012	2×100T

【Intended Use】

Immunoassay for in vitro quantitative determination of cardiac troponin I in human serum and plasma. This assay is intended to auxiliary diagnosis of myocardial infarction.

Cardiac troponin (Tn) was first reported by Cummins et al in 1987 when they diagnosed acute myocardial infarction (AMI)[1,2]. Troponin (Tn) is the main structural contractile protein of myocardium, which is composed of three subunits: troponin C (TnC), troponin T (TnT) and troponin I (TnI)[3]. TnI can be divided into three subtypes: fast skeletal muscle subtype (fTnI), slow skeletal muscle subtype (sTnI) and myocardial subtype (cTnI). However, cTnI was the only one which could be detected in the myocardium when nine months after birth. cTnI has no subtype and is composed of 209 amino acids. More than 40% of the amino acid sequences are heterogenous to other TnI subtypes, and the amino terminal extends a 32-amino acid sequence, so its antigenicity is significantly different from skeletal muscle.

Clinical trials have shown that cTnI can be released into the blood system within hours of myocardial infarction (AMI) or ischemic injury[4,5]. Elevated cTnI levels (above non-AMI sample values) can be detected in serum within 4-6 hours after the onset of chest pain, with the concentration peaking within 8-28 hours and remaining elevated for 3~10 days after the onset of AMI.

【Test Principle】

Sandwich principle. Total duration of assay: 18 minutes.

1st incubation: A sample, biotinylated monoclonal cTnI-specific antibody and monoclonal cTnI-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, and 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 0.75mg/mL: 0.1 M PBS; 0.05% ProClin™ 300.	1×3.0 mL	2×3.0 mL	1×5.0 mL	2×5.0 mL
(RB)	Anti-cTnI-Ab~biotin 0.8 mg/L: 0.05 M MES buffer; 0.05% ProClin™ 300.	1×3.5 mL	2×3.5 mL	1×6.0 mL	2×6.0 mL
(RA)	Anti-cTnI-Ab~Ru(bpy)$_3^{2+}$ 0.63 mg/L: 0.05 M MES buffer; 0.05% ProClin™ 300.	1×3.5 mL	2×3.5 mL	1×6.0 mL	2×6.0 mL
Calibrator (High)	Equine serum, 0.05% ProClin™ 300	1×2.0 mL	2×2.0 mL	1×2.0 mL	1×2.0 mL
Calibrator (Low)	Equine serum, 0.05% ProClin™ 300	1×2.0 mL	2×2.0 mL	1×2.0 mL	1×2.0 mL

The assignment of calibrators value complies strictly with ISO 17511:2020, and this method can be traced back to NIST/SRM 2921.

Supporting instruments and materials required but not provided in this pack (provided by Lifotronic)

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

- Auffer, 480 mL, 6×480 mL
- Buffer, 480 mL, 6×480 mL
- Concentrated Washing Buffer
- Assay Cup

Additional materials for Automated ECL Immunoassay Analyzer eCL9000, eCL9000i, eCL9600, eCL9900, eCL9900i:

- Auffer, 2 L
- Buffer, 2 L
- Concentrated Washing Buffer
- Assay Cup, reaction cup
- Disposable Sampling Head, pipette tips
- PreClean, system wash solution
- Waste Bags, litter bags

【Storage and Shelf Life】

Unopened reagent rackpack, calibrators should be placed at 2~8ºC and will be valid for 12 months.

The reagent can be stored in the machine (4~15°C) for 28 days. Opened reagents should be used and stored at 2~8ºC in 28 days, otherwise trashed.

Opened calibrators should be used and stored at 2~8ºC in 8 hours, or aliquoted into EP tubes and stored at -25~-15ºC in 90 days, otherwise trashed.

The expiration date is labeled on the box, rackpack and bottles.

Damaged, expired or contaminated reagents should be discarded.

【Applicable Instrument】

Automated ECL Immunoassay Analyzer: eCL8000, eCL8000i, eCL8000p, eCL8000x, eCL9000, eCL9000i, eCL9600, eCL9900, eCL9900i.

【Specimen collection, handling and storage】

Human serum and plasma added with heparin lithium, heparin sodium, EDTA-3K and sodium citrate 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 in suggestion. Samples would better to be tested in 8 hours after collection, otherwise, should be stored 2~8ºC for no more than 24 hours or -25~-15ºC, 3 months. Freezing and thawing cycle is permitted only once.

【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 Troponin I (cTnI) test according to the operational manual.

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

The sample volume required for each cTnI test is 100 µL.

Suggested testing environment is 10~30ºC; Relative humidity less than 80%.

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 operational 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) The reagents of different lot are used;

(2) The same lot of reagents were used on the analyzer beyond 28 days;

(3) Quality control misses the target.

Handing of lyophilized calibrators:

Carefully dissolve the contents of one bottle by adding exactly 2.0 mL of distilled or deionized water and allow to stand closed for 10~15 minutes to reconstitute. Mix carefully, avoiding foam formation. Transfer aliquots of the reconstituted calibrators to the additional bottles. After bottles is marked and dispensed. If you do not test immediately, you need to store the aliquots immediately at -25~-15° C

Quality control

In order to ensure the reliability of test results, it's recommended to test the control materials every 24 hours. Assorted Multi Control Cardiac Marker from Lifotronic is bestt recommendated to monitor the reagent. After each calibration, reagent lot change, maintenance or failure repair, quality control is recommended. The quality control results should fall within the scope of local regulations. If beyond, the analyzer status, reagents, calibration and other factors should be checked.

Calculation

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

1 ng/mL = 1 µg/L; 1 ng/mL = 1000 pg/mL

Specimen dilution

Samples with cTnI concentrations above the measuring range can be diluted with cTnI-negative sample diluent. The recommended dilution ratio is 1:10. The

concentration of the diluted sample must be > 3 ng/mL. After manual dilution, multiply the result by the dilution factor.

【Biological reference interval】

According to 312 cases of serum samples of seemingly healthy adult without heart disease human in Guangdong hospitals, P. R. China, the 99 percentile draws a reference range as follow:

Reference range	cases	99 percentile （ng/mL）
10~85 years old	312	0.028

Acute myocardial infarction (AMI) cut-off value

By analyzing 203 patients with chest pain, according to the world health organization (WHO) AMI diagnostic criteria, of which 73 for AMI, we set up cut-off value of AMI 0.1 ng/mL using receiver-operating characteristic (ROC) curve method.

As the differences in geography, race, gender and age, each laboratory should investigate transferability of the reference range to its local population and if necessary determine its own reference ranges.

【Result Interpretation】

In interpreting the results, the patient's overall clinical situation 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.

The measuring range of the kit is 0.006~100 ng/mL. Values below lower detection limit are reported as < 0.006 ng/mL. Values above the measuring range, the sample need be diluted and the results will be reported as the measured value multiplied by the dilution factor.

When samples contain the lipid concentration of 1000 mg/dL or less, bilirubin concentration of 20 mg/dL or less, hemoglobin concentration of 500 mg/dL or less, total protein concentration of 8 g/dL or less, rheumatoid factor concentration of 1500 IU/mL or less, biotin concentration of 20 ng/mL or less, HAMA concentration at 100 ng/mL or less, the interference bias of determination results deviation within ± 15%.

There is no high-dose hook effect at cTnI concentration 2000 ng/mL.

【Analytical Performance】

Lower limit of measurement

The lower detection limit is 0.006 ng/mL (repeatability study, n = 20).

Accuracy

When cTnI of known concentration was added to the matrix without the tested substance, the recovery rate should be within (85%~115%).

Linearity

The correlation coefficient (r) was not less than 0.9900 in the interval of 0.006~100 ng/mL.

Within-run precision (repeatability)

The coefficient of variation (CV) is less than 6%.

Between-lot precision

The coefficient of variation (CV) is less than 10%.

Analytical specificity

Test the following analog-additive blank samples, the cross reaction rate as below.

Analog	Concentration	Cross reaction rate (%)
Sk-TnI	1000 ng/mL	0.001%
cTnC	1000 ng/mL	0.001%
cTnT	1000 ng/mL	0.002%

【Biosafety】

The HBsAg, HIV antibody and HCV antibody test results of calibrators in the kit are all negative. Nevertheless, any test could not exclude absolutely possibility of contagion. Thus, essential and regulation-compliant precautions should be adopted when operating calibrators in the kit.

【Precaution and Warning】

The kit is only used for in vitro diagnostics.

When using the kit, it's necessary to comply with regulations in the laboratory.

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

Test results of the kit are for clinical reference only, and clinical evaluation of patients should be combined with their symptoms and signs, medical history, other laboratory examination results and treatment responses.

Due to factors such as methodology or antibody specificity, testing identical samples with reagents from different manufacturers may get different results, and the results from different kits should not compare directly, lest cause the wrong medical explanation. It's suggested that laboratorians should point out characteristics of the used reagent in the test report to the clinician. In serial monitoring, if the reagent type is changed, a continuous parallel test should be performed and comparison of the results to the former to determine new baseline value.

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. The preservative ProClin™ 300 contains 3% 2-methyl-4-isothiazolin-3-one (MIT) and 5-chloro-2-methyl-4-isothiazolin-3-one (CMIT), will be harmful if inhalation, contact with skin and/or swallow, and may be toxic to aquatic organisms; thus, proper personal protection should be adopt while handling the reagent, and abandoned reagents should be dealed with in compliance with local regulations.

【Symbol】

Symbol	Title of Symbol	Symbol	Title of Symbol
	Manufacturer		Consult instructions for use
	Use by		In vitro diagnostic medical device
	Lot number		Sufficient for <n> tests
	Serial number		Biological risks
	Temperature limitation		This way up
	Authorized representative in the European Community		Indicates this device is in compliance with Europe Directive.
	Catalogue number	P/N	Material code

【Reference】

1. Cummins P, Perry V. Troponin I from human skeletal and cardiac muscles. Biochem J 1978;171:251-259.
2. Wilkinson JM, Grand RJA. Comparison of amino acid sequence of troponin I from different striated muscles. Nature 1978;271:31-35.
3. Mair J, Morandell D, Genser N, et al. Equivalent early sensitivities of myoglobin, creatine kinase MB mass, creatine kinase isoform ratios, and cardiac troponins I and T for acute myocardial infarction. Clin Chem 1995;41;1266-1272.
4. Tanasijevic MJ, Cannon CP, Antman EM. The role of cardiac troponin I (cTnI) in risk stratification of patients with unstable coronary artery disease. Clin Cardiol 1999;22:13-16.
5. Jeffrey L. Anderson, Cynthia D. Adams, Elliott M. Antman, et al. 2011 ACCF/AHA Focused Update of the Guidelines for the Management of Patients With Unstable Angina/Non–ST-Elevation Myocardial Infarction (Updating the 2007 Guideline): A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Circulation 2011.

【Manufacturer】

Shenzhen Lifotronic Technology Co., Ltd.

Unit A, 4th Floor, Building 15, Yijing Estate, No.1008 Songbai Road, Nanshan District, Shenzhen City, Guangdong Province, 518055, P.R.China

E-mail：inter-service@lifotronic.com

【European Representative】

Shanghai International Holding Corp. GmbH (Europe)

Eiffestrasse 80, 20537 Hamburg, Germany

Tel: +49-40-2513175 Fax: +49-40-255726

【Version and Revision】

Version: A3

Issue Date:2021-11-18