

Instruction for Use

【Product Name】

Troponin I (Electrochemiluminescence Immunoassay)

【Package】

100T, 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 with a ruthenium complex react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex binds to the solid phase via interaction of biotin and streptavidin.
- Measurement: The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with Buffer. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via calibration and a master curve provided via the reagent barcode.

【Main Component】

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

Component	Ingredients	Volume (100T)	Volume (2×100T)
(MB)	Streptavidin-coated microparticles 0.75mg/mL; 0.1 M PBS; 0.05% ProClin™ 300.	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.	1×6.0 mL	2×6.0 mL
(RA)	Anti-cTnI-Ab~Ru(bpy) ₂ ³⁺ 0.63 mg/L; 0.05 M MES buffer; 0.05% ProClin™ 300.	1×6.0 mL	2×6.0 mL
Calibrator (High)	Equine serum, 0.05% ProClin™ 300	1×2.0 mL	1×2.0 mL
Calibrator (Low)	Equine serum, 0.05% ProClin™ 300	1×2.0 mL	1×2.0 mL

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The assignment of calibrators value complies strictly with GB/T21415, and this method can be traced back to NIST/SRM 2921.

See the RFID card for the target value of calibrators.

Calibrators are lyophilized which are completely dissolved within 20 minutes after addition of deionized water.

Materials and instruments required but not provided:

- Auffer
- Buffer
- Concentrated Washing Buffer
- eCL8000 series Automated ECL Immunoassay Analyzer
- Assay Cup

【Storage and Shelf Life】

Unopened reagent rackpack, calibrators should be placed at 2~8 °C and will be valid until expiration date.

Opened reagents should be used and stored at 2~8 °C in 28 days, otherwise trashed.

Opened calibrators should be used and stored at 2~8 °C in 8 hours, or aliquoted into EP tubes and stored at -20 °C 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.

【Specimen collection, handling and storage】

Human serum and plasma added with heparin lithium, heparin sodium, EDTA-K₃ 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 2~8 °C for no more than 24 hours or -20 °C, 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.

Suggested testing environment is 10 ~ 30 °C, Relative humidity less than 80%.

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

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.

Pre-treatment of lyophilized calibrators:

Accurately add 2.0 mL deionized water, cover and gently shake to dissolve the lyophilized components completely, stand still vertically for 10~20 min to redissolve the substances at room temperature. Mix well and avoid bubbles. The calibrator should be labeled separately. If the test is not urgent, place the calibrator quickly to store - 20 °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 best recommended to monitor the reagent. After each calibration, reagent lot change, maintenance or failure repairment, 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

The relative bias of measuring trueness control materials should fall between 0.85 and 1.15.

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 dealt with in compliance with local regulations.

【Symbol】

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

【Reference】

- Cummins P, Perry V. Troponin I from human skeletal and cardiac muscles. *Biochem J* 1978;171:251-259.
- Wilkinson JM, Grand RJA. Comparison of amino acid sequence of troponin I from different striated muscles. *Nature* 1978;271:31-35.
- 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.
- 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.
- 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.

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