BIO TECHNOLOGY boditech

Cardiac

ichroma™ Tn-I

INTENDED USE

ichroma™ Tn-I is a Fluorescence Immunoassay (FIA) for the quantitative determination of cardiac troponin-I (Tn-I) in human serum/plasma. It is useful as an aid in management and monitoring of acute myocardial infarction (AMI).

For *in vitro* diagnostic use only.

INTRODUCTION

Cardiac troponins are currently the most sensitive and specific biochemical markers of myocardial necrosis. There are three types of troponin in heart muscle fibers. Those are troponin-C, troponin-I, and troponin-T. Together they contribute to make cardiac muscle fibers contract. The clinical measurement of serum Tn-I has become an important tool in the diagnosis of acute myocardial infarction. Serum Tn-I is a more reliable than creatine kinase as a prognostic marker in people with ischemic chest pain. National and international scientific organizations have suggested the use of troponins, Tn-I and Tn-T, when implementing new diagnostic strategies in patients with acute coronary syndrome.

PRINCIPLE

The test uses a sandwich immunodetection method; the detector antibody in buffer binds to antigen in sample, forming antigen-antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-antibody on test strip.

The more antigen in sample forms the more antigen-antibody complex and leads to stronger intensity of fluorescence signal on detector antibody, which is processed by instrument for ichroma™ tests to show Tn-I concentration in sample.

COMPONENTS

ichroma™ Tn-I consists of 'Cartridges', 'Detection Buffer Vial', 'Sample Mixing Tubes' and an 'ID chip'.

- The cartridge contains a test strip, the membrane which has anti human Tn-I at the test line, while streptavidin at the control line.
- Each cartridge is individually sealed in an aluminum foil pouch containing a desiccant. 25 sealed cartridges are packed in a box which also contains an ID chip.
- The detection buffer contains anti human Tn-I-fluorescence conjugate, biotin-BSA-fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide as a preservative in phosphate buffered saline.
- The detection buffer is dispensed in a vial. Detection buffer vial is packaged in a Styrofoam box with ice-pack for the shipment.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Carefully follow the instructions and procedures described in this 'Instruction for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge, ID chip and detection buffer) must match each other.
- Do not interchange the test components between different lots or use the test components after the expiration date, either of which might yield misleading of test result(s).
- Do not reuse. A sample mixing tube should be used for processing one sample only. So should a cartridge.
- The cartridge should remain sealed in its original pouch before use. Do not use the cartridge, if is damaged or already opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with the regulations. Sample with severe hemolytic and hyperlipidemia cannot be used and should be recollected.
- Just before use, allow the cartridge, detection buffer and sample to be at room temperature for approximately 30 minutes.
- **ichroma™ Tn-I** as well as the instrument for ichroma™ tests should be used away from vibration and/or magnetic field. During normal usage, it can be noted that instrument for ichroma™ tests may produce minor vibration.
- Used sample mixing tubes, pipette tips and cartridges should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- An exposure to larger quantities of sodium azide may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure.7
- **ichroma™ Tn-I** will provide accurate and reliable results subject to the following conditions.
  - Use **ichroma™ Tn-I** should be used only in conjunction with instrument for ichroma™ tests.
  - Any anticoagulants other than **heparin, sodium citrate** should be avoided.

STORAGE AND STABILITY

- The cartridge is stable for 20 months (while sealed in an aluminum foil pouch) if stored at 4-30 °C.
- The detection buffer dispensed in a vial is stable for 20 months if stored at 2-8 °C.
- After the cartridge pouch is opened, the test should be performed immediately.

LIMITATION OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result. The non-responsiveness of the antigen to the antibodies is most common where the epitope is masked by some unknown components, so as not to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may cause the false negative as it makes antigen unrecognizable by the antibodies.
- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or presence of interfering substances in the test samples.
- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician including clinical symptoms and other relevant test results.

MATERIALS SUPPLIED

REF 13011

Components of **ichroma™ Tn-I**

- Cartridge Box:
  - Cartridges 25
  - ID Chip 1
  - Instruction For Use 1
  - Sample Mixing Tubes 25
- Detection Buffer Vial 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **ichroma™ Tn-I**. Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - **ichroma™ Reader** REF FR203
  - **ichroma™ II** REF FPRR021
  - **ichroma™ D** REF 13303
- **ichroma™ Printer** REF FPRR007
- **Boditech Cardiac Control** REF CFPO-98

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ Tn-I** is human serum/plasma.

- It is recommended to test the sample within 24 hours after collection.
- The serum or plasma should be separated from the clot by centrifugation within 3 hours after the collection of whole blood. If longer storage is required, e.g. if the test could not be performed within 24 hours, serum or plasma should be immediately frozen below -20 °C. The freezing storage of sample up to 3 months does not affect the quality of results.
- Once the sample was frozen, it should be used one time only for test, because repeated freezing and thawing can result in the change of test values.

TEST SETUP

- Check the contents of **ichroma™ Tn-I**: Sealed Cartridge, Detection Buffer Vial, Sample Mixing Tubes and ID Chip.
- Ensure that the lot number of the cartridge matches that of the ID chip as well as the detection buffer.
- Keep the sealed cartridge (if stored in refrigerator) and the detection buffer tube at room temperature for at least 30 minutes just prior to the test. Place the cartridge on a clean, dust-free and flat surface.
- Turn on the instrument for ichroma™ tests.
- Insert the ID Chip into the ID chip port of the instrument for ichroma™ tests.
- Press the 'Select' button on the instrument for ichroma™ tests. (Please refer to the 'Instrument for ichroma™ tests Operation Manual' for complete information and operating instructions.)

TEST PROCEDURE

1) Transfer 75 µL (Human serum/plasma/control) to an empty sample mixing tube using a transfer pipette and add 75 µL detection buffer to it.
2) Close the lid of the sample mixing tube and mix the sample thoroughly by shaking it about 10 times. (The sample mixture must be used immediately.)
3) Pipette out 75 µL of a sample mixture and load it into the sample well on the cartridge.
4) Leave the sample-loaded cartridge at room temperature for 12 minutes.
   ⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inexact test result.*
5) To scan the sample-loaded cartridge, insert it into the cartridge holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow has been marked on the cartridge especially for this purpose.
6) Press 'Select' button on the instrument for ichroma™ tests to start the scanning process.
7) Instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.
8) Read the test result on the display screen of the instrument for ichroma™ tests.

INTERPRETATION OF TEST RESULT

- **Result**
  - Alternate Result Unit**:** The default result unit for ichroma™ Tn-I is ng/mL. When selecting the alternate result unit, µg/L, the conversion factor used by the ichroma system is 1.0. The conversion formula to change to the alternate result unit is: ng/mL x 1.0 = µg/L

- **Limits and Ranges**
  - Working range: 0.10–50 ng/mL
  - Lower limits of measurement:

Limit of Blank (LoB):	0.07 ng/mL
Limit of Detection (LoD):	0.11 ng/mL
Limit of Quantitation (LoQ):	0.30 ng/mL

  - The LoB and LoD were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.
  - The LOB is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series.
  - The LoD is determined based on the LoB and the standard deviation of low concentration samples.
  - The LoQ (functional sensitivity) is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

- **Expected Values**
  - In studies performed with the **ichroma™ Tn-I** assay involving 100 healthy volunteers in Korea, the upper reference limit (99th percentile) for Tn-I was 0.11 ng/mL. The lowest concentration with a CV less than or equal to 10 % with the **ichroma™ Tn-I** assay was 0.50 ng/mL.
  - Due to the release kinetics of Tn-I, a result below the decision limit within the first hours of the onset of symptoms does not rule out myocardial infarction with certainty. If myocardial infarction is still suspected, repeat the test at appropriate intervals.
  - A cut-off of 0.3 ng/mL Tn-I is recommended for diagnosis of AMI, as this yields optimal performance of 91 % of sensitivity and 92.1 % of specificity. However, laboratories should establish their own diagnostic cut-off concentration based on the clinical practice at their perspective institutions.

QUALITY CONTROL

- Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
- The control tests should be performed immediately after opening a new test lot to ensure the test performance is not altered.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are not provided with **ichroma™ Tn-I**. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance. (Please refer to the instruction for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Specificity**: There, in test samples, are biomolecules such as as heparin, protein kinase A (PKA), creatine kinase, autoantibodies, and free and binary or ternary troponin complex were added to the test sample(s) at concentrations much higher than their normal physiological levels in blood. **ichroma™ Tn-I** test results did not show any significant cross-reactivity with these biomolecules.
- **Precision**: The intra-assay precision was calculated by one evaluator, who tested different concentration of control standard ten times each with three different lots of **ichroma™ Tn-I.** The inter-assay precision was confirmed by 3 different evaluators with

3 different lots, testing three times each different concentrations.

Tn-I (ng/mL)	Intra-assay		
	Mean	SD	CV (%)
0.20	0.18	0.03	22.77
0.40	0.39	0.02	8.31
2.70	2.65	0.07	4.96
9.00	9.02	0.19	2.79
26.00	25.99	0.76	2.14

Tn-I (ng/mL)	Inter-assay		
	Mean	SD	CV (%)
0.20	0.20	0.04	38.40
0.40	0.38	0.03	18.27
2.70	2.58	0.13	5.53
9.00	9.02	0.27	3.00
26.00	26.00	0.68	2.6

- **Diagnostic sensitivity and specificity**

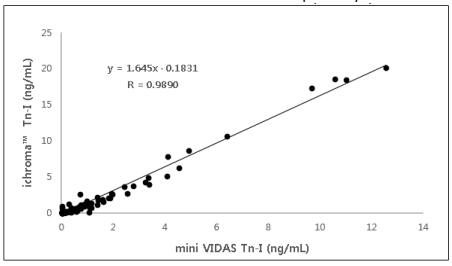
  A total of 122 serum/plasma samples, 46 positive and 76 negative, were tested a commercially available troponin I assay. The sample were tested by ichroma™ instrument. The sample concentrations were between approx. 0.10 and 18.44 ng/mL.

  A Receiver Operating Characteristic (ROC) curve was calculated from the peak troponin values.

  Calculation of the peak values of the commercially available cardiac troponin I test measured in parallel yielded the following results for the officially stated ROC optimized cut-off of 0.30 - 0.50 ng/mL.

Cut-off µg/l (ng/mL)	Sensitivity (%)	N	95% CI (%)	Specificity (%)	N	95% CI (%)
0.30	91.3	42/46	79.2~97.6	92.11	70/76	83.6~97.0
0.50	78.26	36/46	63.6~89.1	94.74	72/76	85.3~97.8

- **Comparability:** Tn-I concentrations of 150 clinical samples were quantified independently with **ichroma™ Tn-I** and mini VIDAS (BioMerieux Inc. France) as per prescribed test procedures. Test results were compared and their comparability was investigated with linear regression and coefficient of correlation (R). Linear regression and coefficient of correlation between the two tests were Y= 1.645 X – 0.1831 and R = 0.9890 respectively.



REFERENCES

1. Mauro Panteghini, Franca Pacani, Kiang-Teck J.Yeo, Fred S. Apple, Robert H. Christenson. Francesco Dati, Johannes Mair, Jan Ravkilde, and Alan H.B. We. Evaluation of Imprecision for Cardiac Troponin Assays at Low-Range Concentrations. 2004;50:2:327-332.
2. Alan McNeil, PhD, FRACP, FRCPA. The Trouble with Troponin. Heart, Lung and Circulation 2007;16;S13-S16.
3. David M. Bunk and Micahel J. Welch. Characterization of a New Certified Reference Material for Human Cardiac Troponin I. Clinical Chemistry 2002;52:2:212-219
4. Jaffe AS, Ravkilde J, Roberts R, Naslund U, Apple FS, Galvani M, Katus H. It's time for a change to a troponin standard. Circulation 2000;102:1216 –1220.
5. Jillan R. Tate, David Heathcote, Gus Koerbin, Gary Thean, David Andriske, Jone Bonar, Janice Gill. Theharmonization of cardiac troponin I measurement is independent of sample time collection but is dependent on the source of calibrator. Clinica Chimica Acta 324:2002:13-23.
6. Ohman EM, Armstrong PW, Christenson RH, et al. Cardiac troponin T levels for risk stratification in acute myocardial ischemia. N Engl J Med 1996;335:1333– 41.
7. Antman EM, Tanasijevic MJ, Thompson B, et al. Cardiacspecific troponin I levels to predict the risk of mortality in patients with acute coronary syndromes. N Engl J Med 1996;335:1342 –9.

Note: Please refer to the table below to identify various symbols

Σ	Sufficient for <n> tests
	Read instruction for use
	Use by Date
LOT	Batch code
REF	Catalog number
	Caution
	Manufacturer
EC REP	Authorized representative of the European Community
IVD	In vitro diagnostic medical device
	Temperature limit
	Do not reuse
CE	This product fulfills the requirements of the Directive 98/79/EC on in vitro diagnostic medical devices