# CARDIAC-SPECIFIC TROPONIN-I (cTn-I)

**INTENDED USE**

For the quantitative determination of Cardiac-Specific troponin-I (cTn-I) in Human Serum

**INTRODUCTION**

Troponin I is the regulatory subunit of the troponin complex associated with the actin thin filament within muscle cells[1]. In striated muscles, both skeletal and cardiac, it forms a protein complex with troponin T and troponin C, together the complex functions in the regulation of muscle contraction[3]. Recent clinical studies have shown the dissociation of the troponin complex following myocardial damage, and the release of individual protein components into the bloodstream. Although troponin I is also found in skeletal muscles, and may be released after extensive physical stress, this form differs from cTnI in its amino acid composition by approximately 40%[2]. This distinction allows the two forms of troponin I to be distinguished immunologically and thereby ensures an accurate test assay that is specific to only cardiac troponin I molecules[1,3].

Elevated levels of cardiac troponin I (cTnI) can be detected in the blood circulation soon after the onset of cardiac damage.[4-6] While the normal serum level of cTnI is below 0.06ng/ml, a level of cTnI greater than 1.5 ng/ml can be detected by Troponin I ELISA Test in as soon as 4 to 6 hours following an acute myocardial infarction (AMI). cTnI levels can reach peak concentrations as high as 100-1300ng/ml in some AMI patients in approximately 12 to 24 hours after infraction, and may remain elevated for 3 to 10 days.[7-10] The extended elevation makes cTnI a useful marker in the differential diagnosis of patients presenting to Emergency Departments with chest pain. Its release pattern is similar to CK-MB (4-6 hours after onset of pain), but provides a longer temporal window of detection of cardiac injury[18]. Its extended elevation along with its diagnostic sensitivity and cardiac specificity allows for the detection of AMI as early as 4 hours after the onset of ischemia.

While cTnI can be used in diagnosis of peri-operative infarction in situations where a high serum level of skeletal muscle proteins are expected, recent data have also identified a measurable relationship between cTnI levels and long-term outcome after an episode of chest discomfort[16]. These studies suggest that the quantitative determination of cTnI reveals high predictive value in diagnosing the high risk group of unstable angina patients, and that such tests may be particularly useful in evaluating patient condition prior to discharge from the ED[16,19,20].

The Cardia-Specific Troponin I ELISA Test is a quantitative enzyme immunoassay that provides a rapid, sensitive, and reliable assay for the quantitative measurement of cardiac-specific Troponin I. The test kit can be used together with other diagnostic methods to assess cardiac damage caused by AMI.

**PRINCIPLE OF THE TEST**

The cTnI EIA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes micro titer wells coated with monoclonal anti-TnI antibodies. Two other monoclonal antibodies are in the antibody-horseradish peroxidase (HRP) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, and the troponin I molecules are sandwiched between the immobilized and enzyme-linked antibodies. After 15-minute incubation, the wells are washed to remove unbound labeled antibodies, and a substrate/chromogen solution is added. The reaction between HRP and the substrate/chromogen mixture result in the development of a blue color, which is stopped with the addition of stop solution, changing the solution to yellow. The concentration of troponin-I is directly proportional to the color intensity of the test sample, which is measured spectrophotometrically at 450mm.

**MATERIALS AND COMPONENTS**

***Materials provided with the test kit:***

- Antibody-coated -microtiter wells 96 wells
- Enzyme Conjugate Reagent 12 ml
- TMB Substrate 12 ml
- Stop Solution 12 ml
- Set of Reference Standards: 0.5ml each
  0, 0.5, 2, 4, 8 and 16 ng/ml in lyophilized form
- Wash buffer Concentrate(50X) 15ml
- Control Set, lyophilized form 0.5 ml

**WARNING AND PRECAUTIONS**

1. CAUTION: The Positive and Negative Controls for use with this kit contain human material. The source material used for manufacture of these components tested negative for HBsAg, HIV 1/2 and HCV by FDA-cleared methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. It is recommended that the reagents and patient samples be handled according to the OSHA Standard on Blood-borne Pathogens[11] or other appropriate national biohazard safety guidelines or regulations.[12-13]
2. Do not use beyond the expiration date indicated on the product and do not mix or use components from kits with different lot numbers.
3. For in vitro diagnostic use only.
4. Avoid contact with stop solution (HCl). It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
5. Use separate clean tips for different specimens. Do not pipette by mouth.
6. Replace caps on reagents immediately after use and do not switch caps.
7. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
8. Wear disposable gloves while handling specimens and thoroughly wash hands afterwards.
9. All patient samples should be handled as if they are capable of transmitting diseases. Observe established procedures for proper disposal of specimens and used test devices.
10. After reconstitution, all Troponin I standards should be stored at -20 $^0$C when not in use. For longer peroid of storage time, aliquot the reconstituted standards in polypropylene tubes and store in -20ºC. **Do not freeze and thaw more than once.**

**SPECIMEN COLLECTION AND PREPARATION**

1. The use of serum samples is required for this test.
2. Specimens should be collected using standard venipuncture techniques. Remove serum from the coagulated or packed cells within 60 minutes after collection.
3. Specimens which cannot be assayed within 24 hours of collection should be frozen at -20ºC or lower, and will be stable for up to six months.
4. Avoid hemolytic (bright red), or turbid samples.
5. Specimens should not be repeatedly frozen and thawed prior to testing. Specimens must be mixed thoroughly after thawing, by low speed vortexing or by gently inverting, and centrifuged prior to use to remove particulate matter and to ensure consistency in the results.

**STORAGE AND HANDLING**

6. All reagents should be refrigerated at 2-8ºC. Return all reagents requiring refrigeration to 2-8ºC immediately after use.
7. Microtiter wells should be kept in a sealed bag with a desiccant.
8. Bring sealed wells to room temperature before opening zip-lock bag to avoid condensation build-up in wells or bags.
9. After removing the desired number of wells, keep the desiccant in the zip-lock bag at all times.
10. All reagents are stable up to their expiration dates shown when stored at 2-8ºC.
11. Wear disposable glove while handling specimens and thoroughly wash hands afterwards. All patient samples should be handled as if they are potentially infectious. If serum or plasma samples have been stored in the refrigerator, allow them to return to room temperature before testing.

**REAGENT PREPARATION**

1. All reagents should be brought to room temperature (18-22°C) before use.
2. If reference standards are lyophilized, reconstitute each standard with 0.5 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards are stable for up to 20 days when stored sealed at 2-8°C. For longer peroid of storage time, aliquot the reconstituted standards in polypropylene tubes and store in -20ºC. Do not freeze and thaw more than once.
3. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, dilute 15 ml of Wash Buffer (50x) into 735 ml of distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.

**ASSAY PROCEDURE**

1. Secure the desired number of coated wells in the holder.
2. Dispense 25µl of standards, specimens, and controls into appropriate wells.
3. Dispense 100µl of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to have a complete mixing in this step.
5. Incubate at room temperature (18-22°C) for 15 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Rinse and flick the microtiter wells 5 times with wash buffer (1x).
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100µl of TMB solution into each well. Gently mix for 5 seconds.
10. Incubate at room temperature for 15 minutes.
11. Stop the reaction by adding 100µl of Stop Solution to each well.
12. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
13. Read optical density at 450nm with a microtiter well reader.

**IMPORTANT NOTE**

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

**QUALITY CONTROL**

Good laboratory practice requires that quality control specimens (controls) be run with each calibration curve to verify assay performance. To ensure proper performance, control material should be assayed repeatedly to establish mean values and acceptable ranges.

**CALCULATION OF RESULTS**

1. Calculate the mean optical density value (OD450) for all sets of the reference standards, unknown samples and blanks
2. Subtract the average blank value from the average values of the reference standards and unknown samples.
3. Construct a standard curve for the reference standards by plotting each of the Troponin I concentrations in ng/mL on the X-axis and their corresponding OD450 values on the Y-axis.
4. Using the mean absorbance value for each unknown sample, determine the corresponding concentration of troponin I (ng/ml) from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data analysis may be employed.
5. Patient samples with cTnI concentrations greater than 30ng/ml should be diluted with Sample Diluent (0ng/mL cTnI Standard). The final cTnI values should be multiplied by the dilution factor to obtain cTnI results in ng/ml.

**EXAMPLE OF STANDARD CURVE**

Results of a typical standard run with optical density reading at 450nm shown in the Y axis against cTnI concentrations shown in the X axis.

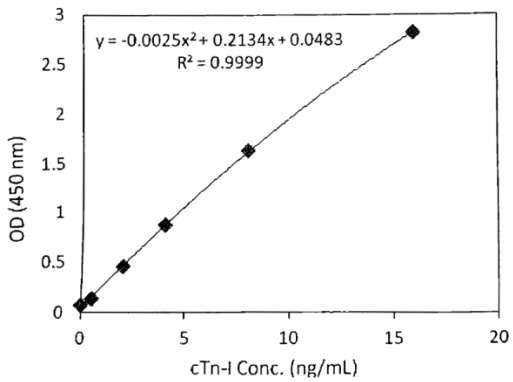
**This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns.**

| cTnI (ng/ml) | Absorbance (450nm) |
|---|---|
| 0.0 | 0.068 |
| 0.5 | 0.141 |
| 2.0 | 0.455 |
| 4.0 | 0.859 |
| 8.0 | 1.602 |
| 16.0 | 2.816 |

**PRESENTACIÓN:**

CONT. 96 TEST CODIGO: RSET030

# CARDIAC-SPECIFIC TROPONIN-I (cTn-I)




**EXPECTED VALUES**

It is recommended that each laboratory establish its own normal range based on the patient population, geography, dietary and environmental factors, and to reflect current practice and criteria for AMI-diagnosis.

An evaluation of the clinical data was conducted to determine the normal expected value, as well as the clinical sensitivity and clinical specificity of the Troponin I ELISA Test. Serum samples from 80 apparentenly healthy individuals were assayed. The 0-97.5% of the results spanned 0.00 ng/ml to 1.00 ng/ml. The 0-99% of the results spanned 0.00 ng/ml to 0.06 ng/ml. **These ranges should be used as guidelines only. Each laboratory should establish its own reference ranges.**

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

Any conditions resulting in myocardial cell damage can potentially increase cardiac troponin-I levels above the expected value. These conditions have been documented clinically to include unstable angina, myocarditis, congestive heart failure, and cardiac surgery or invasive testing[16,17].

**PERFORMANCE CHARACTERISTICS**

1. Precision

Intra-assay precision

| Serum Sample | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| # Replicates | 10 | 10 | 10 | 10 | 10 |
| Mean cTnI (ng/ml) | 1.18 | 2.13 | 4.47 | 9.28 | 21.3 |
| S.D. | 0.039 | 0.043 | 0.16 | 0.624 | 0.734 |
| CV (%) | 3.27 | 2.02 | 3.58 | 6.78 | 3.45 |

2. Sensitivity

The minimal detectable concentration of cTn-I by this assay is estimated to be 0.04ng/ml.

3. Interference and Cross-Reactivity

The following were tested for cross-reactivity at concentrations up to the levels indicated below. No cross-reactivity was observed for any of the components.

| ANALYTE | TEST CONCENTRATION |
|---|---|
| Rabbit skeletal muscle troponin C | 2,500 ng/ml |
| Human cardiac troponin T | 2,500 ng/ml |
| Human skeletal muscle troponin T | 2,500 ng/ml |
| Human skeletal muscle troponin I | 2,500 ng/ml |
| Hemoglobin | 200 mg/ml |
| Biotin | 200 ng/ml |
| Bilirubin | 1 mg/ml |

The following materials commonly found in serum specimens exhibited no interference with test results at levels below the specified concentrations:

| Analyte | Concentration |
|---|---|
| Heparin | 14 IU/mL |
| Warfarin | 10 ug/mL |
| EDTA | 18 mg/mL |
| Red Blood Cells | < 100 per mL |
| Hemolysate | < 0.05% |
| Total proteins | 30mg/mL |

4. Hook Effect

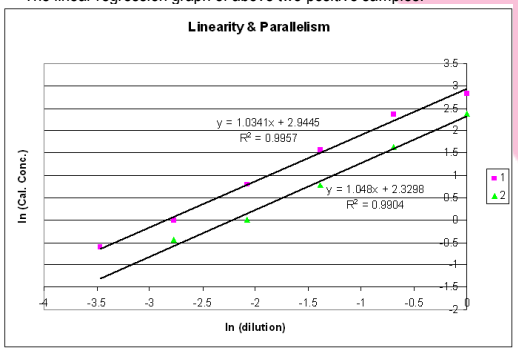
It has been demonstrated at Troponin I levels up to 20,000 ng/ml, this EIA Kit will produce a concentration measurement above 30 ng/mL, which is the upper limit of its linear range. However in view of the limitation of optical measurements in our EIA system, absence of the Hook effect cannot be clearly demonstrated beyond the O.D. reading of 3.000. It is recommended that appropriate sample dilutions be made so that accurate troponin I concentrations can be determined through the precise reading within the linear range of this EIA system. For any sample that either: produces an O.D. reading above 3.0, has a measured concentration above 30ng/mL or is clinically suspected to contain Troponin I level in excess of 30ng/mL, we recommend diluting patient samples before further analysis.

5. Linearity and Parallelism study

A study was conducted to demonstrate linearity of the assay. Two positive patient samples were serially diluted. ng/ml values were calculated for individual OD readings of the diluted samples. The linearity of $R^2$ values and slope values are listed in the following table:

| Sample | Neat | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | slope | $R^2$ |
|---|---|---|---|---|---|---|---|---|
| 1 | 17.0 | 10.5 | 4.72 | 2.21 | 0.99 | 0.54 | 1.034 | 0.99 |
| 2 | 10.9 | 5.16 | 2.17 | 1.02 | 0.65 | 0.40 | 1.048 | 0.99 |

The linear regression graph of above two positive samples:




**LIMITATIONS OF THE PROCEDURE**

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. Troponin-I levels can be increased in any conditions resulting in myocardial cell damage. Diagnostic results obtained from this assay should be used in conjunction with other diagnostic procedures and information available to the physician such as addition clinical testing, ECG, symptoms, and clinical observations.
3. Serum samples demonstration gross hemolysis, lipemia, or turbidity should not be used with this test.
4. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
5. Patient samples may contain human anti-mouse antibodies (HAMA) which are capable of giving falsely elevated or depressed results with assays that utilize mouse monoclonal antibodies. Reliable results in specimens with HAMA levels above 50µg/mL cannot be guaranteed in these specimens, and all test results should be used in conjunction with additional clinical observations.
6. Test results that are inconsistent with the clinical picture and patient history should be interpreted with caution.
7. In view of the limitation of optical measurements in our EIA system, absence of the Hook effect cannot be clearly demonstrated beyond the O.D. reading of 3.000. It is recommended that appropriate sample dilutions be made so that accurate troponin I concentrations can be determined through the precise reading within the linear range of this EIA system.

**REFERENCES**


1. Perry SV: The regulation of contractile activity in Muscle. Biochem Soc Trans 1979; 7:593
2. William JM, Grand RJA: Comparison of amino acid of troponin I from different striated muscles. Nature 1978; 271:31
3. Hartner KT, Petle D:Fast and slow isoforms of troponin I and troponin C. Distribution in normal rabbit muscles and effects of chronic stimulation. Eur J Biochem 1990; 188:261
4. Wu AHB, ed: Cardiac Markers. Totowa, NJ, Human Press, 1998, pp 300
5. Hanfer S et al: Cardiac troponins in serum in chronic renal failure. Clin Chem 1994; 40:1790
6. Bhayana V et al: Disordence between results of serum troponin T and I. Clin Chem 1995; 41:312
7. Kateukha AG, Bereznikova AV, Esakova T, Petterson K, Lovgron T, Severina ME: Troponin I is released in blood stream of patients with acute myocardial infarction not in free form but as complex. Clin Chem 1997; 43:1379-85