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. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
4. 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. The vender's cTnI ELISA assay has been designed to minimize interference from HAMA-containing specimens; nevertheless, complete elimination of this interference from all specimens cannot be guaranteed.

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Troponin I ELISA

Catalog No. **IB19116**N (96 Tests)

INTENDED USE

The **IBL-America** cardiac Troponin I (cTnI) ELISA is intended for the determination of cardiac Troponin I in human serum. **For research use only, not for use in diagnostic procedures.**

SUMMARY AND EXPLANATION

Troponin is the inhibitory or contractile regulating protein complex of striated muscle. It is located periodically along the thin filament of the muscle and consists of three distinct proteins: troponin I, troponin C, and troponin T. Likewise, the troponin I subunit exists in three separate isoforms; two in fast-twitch and slow-twitch skeletal muscle fibers, and one in cardiac muscle. The cardiac isoform (cTnI) is about 40% dissimilar, has a molecular weight of 22,500 daltons, Cardiac troponin I (cTnI) has been useful in the differential diagnosis of **subjects** presenting to Emergency Departments (ED) with chest pain 18-20. Myocardial infarction is diagnosed when blood levels of sensitive and specific biomarkers, such as cardiac troponin, the MB isoenzyme of creatine kinase (CK-MB), and myoglobin, are increased in a clinical setting of acute ischemia. The most recently described and preferred biomarker for myocardial damage is cardiac troponin (I or T). The cardiac troponins exhibit myocardial tissue specificity and high sensitivity. The level of cTnI remains elevated for a much longer period of time (6-10 days), thus providing for a longer window of detection of cardiac injury. Normal levels of cTn I in the blood are very low. After the onset of an AMI, cTnI levels increase substantially and are measurable in serum within 4 to 6 hours, with peak concentrations reached in approximately 12 to 24 hours after infarction. The cTnI Enzyme Immunoassay provides a rapid, sensitive, and reliable assay for the measurement of cardiac-specific troponin I. The antibodies developed for the test will determine a minimal concentration of 1.0 ng/ml, and theres no cross-reactivity with human cardiac or skeletal troponin T or I.

PRINCIPLE OF ASSAY

The cTnI ELISA is based on solid phase sandwich ELISA method. The assay utilizes four monoclonal antibodies which recognize different and distinct Troponin I epitopes. The samples and conjugate reagent (anti-cTnI biotin & HRP) are added to the wells coated with Streptavidin. cTnI in the serum binds to the Abs, forming a sandwich complex and simultaneously the complex is being immobilized on the plate through streptavidin-biotin interactions. Unbound protein and conjugate are washed off, through a washing step. Upon addition of the substrate, the intensity of color is proportional to the concentration of cTnI in the samples. A standard curve is prepared by relating the color intensity to the concentration of cTnI.

	Materials Provided	96 Tests
1.	Microwells coated with Streptavidin	12x8x1
2.	cTnI Standard: 6 vials (Lyophilized)	1ml
3.	cTnI Conjugate Reagent: 1 bottle (ready to use)	12 ml
4.	TMB Substrate: 1 bottle (ready to use)	12ml
5.	Stop Solution: 1 bottle (ready to use)	12ml
6.	20X Wash concentrate: 1 bottle	25ml

MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450 nm
5. Absorbance paper or paper towel
6. Graph paper

STORAGE AND STABILITY

1. Store the kit at 2-8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light.

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials:
   The calibrator and controls contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984.
2. This kit is designed for **research use only, not for use in diagnostic procedures**.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. It is recommended that standards, control and serum samples be run in duplicate.
6. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION AND PREPARATION

1. Collect blood specimens and separate the serum immediately.
2. Specimens which cannot be assayed within 24 hours of collection should be frozen at –20°C or lower, and will be stable for up to six months.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

REAGENTS PREPARATION

1. **Standards:** Reconstitute each lyophilized standard with 1.0 ml distilled water. The reconstituted standards are stable for 24 hours when stored sealed at 2-8°C. To assure maximum stability of the reconstituted standards, aliquot the standards and store at -20°C. Do not freeze-thaw more than once.
2. Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

ASSAY PROCEDURE

Prior to assay, allow reagents to stand at room temperature.
Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder
2. Pipet 50 µl of cTnI standards, control and sera onto appropriate wells.
3. Add 100 µl of cTnI conjugate reagent to all wells.
4. Cover the plate and incubate for 60 minutes at room temperature (20-25°C).
5. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.
6. Add 100 µl of TMB substrate to all wells.
7. Incubate for 15 minutes at room temperature.
8. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

CALCULATION OF RESULTS

The standard curve is constructed as follows:

1. Check cTnI standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached**.**
2. To construct the standard curve, plot the absorbance for the cTnI standards (vertical axis) versus the cTnI standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.
4. Value above the highest point of the standard are retested after diluting with “0” standard.

Example of a Standard Curve

Results of a typical standard run with absorbency readings at 450nm on the Y axis against troponin I concentrations shown on the X axis. NOTE: This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each laboratory must provide its own data and standard curve in each experiment.

cTnI (ng/ml)	Absorbance (450nm)
0	0.006
1	0.055
3	0.215
6	0.520
18	1.361
36	2.176

EXPECTED VALUES

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population.