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2016-06-08

Cat#: TI015C (96 Tests)
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CALBIOTECH
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Troponin I ELISA

Catalog No. TI015C (96 Tests)

INTENDED USE

For Research Use Only. Not for use in diagnostic procedures.

Materials Provided		96 Tests
1.	Microwells coated with mouse Anti-TnI	12x8x1
2.	Reference Standard Set	1 ml
3.	cTnI Enzyme Conjugate Reagent	13 ml
4.	TMB Reagent	11 ml
5.	Stop Solution	11 ml
6.	Wash Concentrate 20x: 1 Bottle	25 ml

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. For Research Use Only. Not for use in diagnostic procedures.
2. For Laboratory Use.
3. 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.
4. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
5. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
6. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
7. Control sera and sample diluent contain preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

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 –20°C or lower, and will be stable for up to six months.
4. Avoid grossly hemolytic (bright red), lipemic (milky), or turbid samples (after centrifugation).
5. Specimens should not be repeatedly frozen and thawed prior to testing. DO NOT store in “frost free” freezers, which may cause occasional thawing. Specimens which have been frozen, and those which are turbid and/or contain particulate matter, must be centrifuged prior to use.

REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (20-25°C) before use.
2. Reconstitute each lyophilized standard with 1.0 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. The Reconstituted standards will be stable for up to 8 hours when stored sealed at 2-8°C. Discard the reconstituted Standards after 8 hours. To assure maximum stability of the reconstituted Standards, they should be aliquoted and frozen (–20°C or below) immediately after reconstitution has been achieved. Each aliquoted Standard should be frozen and thawed only once.
3. 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

1. Secure the desired number of coated wells in holder.
2. Dispense 100 µl of standards, specimens, and controls into appropriate wells.
3. Dispense 100 µl of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to mix completely.
5. Incubate at room temperature (20-25°C) for 90 minutes.
6. Remove the incubate mixture by flicking plate contents into a waste container.
7. Remove liquid from all wells. Wash wells three times with 300 µL of 1X wash buffer. Blot on absorbance paper or paper towel.
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100 µl of TMB Reagent into each well. Gently mix for 5 seconds.
10. Incubate at room temperature for 20 minutes.
11. Stop the reaction by adding 100 µl 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 absorbance at 450nm with a microtiter well reader within 15 minutes.

CALCULATIONS OF RESEULTS

1. Calculate the mean absorbance value (OD450) for each set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each 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 reduction may be employed.
4. Patient samples with cTnI concentrations greater than 100 ng/ml should be diluted 10-fold with vender’s Troponin I Sample Diluent. The final cTnI results in ng/ml.

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	0.048
2.0	0.110
7.5	0.307
30	1.357
75	2.853

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.