TROPONIN I (HUMAN CARDIAC-SPECIFIC) ENZYME IMMUNOASSAY TEST KIT
Catalog Number: OKBA00025

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Enzyme Immunoassay for the Quantitative Determination of Cardiac-Specific Troponin-I in Human Serum

FOR RESEARCH PURPOSES ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

INTENDED USE
The cTnI ELISA is intended for the quantitative determination of cardiac troponin I in human serum. Measurement of troponin I values are useful in the evaluation of acute myocardial infarction (AMI).

PRINCIPLE OF THE ASSAY
The cTnI ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes four unique monoclonal antibodies directed against distinct antigenic determinants on the molecule. Three mouse monoclonal anti-troponin I antibodies are used for solid phase immobilization (on the microtiter wells). The fourth antibody is in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the four antibodies, resulting in the troponin I molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 90-minute incubation at room temperature, the wells are washed with water to remove unbound-labeled antibodies. A solution of tetramethylbenzidine (TMB) Reagent is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 1N hydrochloric acid (HCl) changing the color to yellow. The concentration of troponin I is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

REAGENTS AND MATERIALS PROVIDED
1. *Antibody-Coated Wells (1 plate, 96 wells)*
   Microtiter wells coated with mouse monoclonal anti-TnI.
2. *Reference Standard Set (1 set, 1.0 ml/vial)*
   Contains 0, 2.0, 7.5, 30, and 75 ng/ml TnI, lyophilized.
3. *cTnI Enzyme Conjugate Reagent (13 ml/vial)*
   Contains mouse monoclonal anti-TnI conjugated to horseradish peroxidase in Tris Buffer-BSA solution with preservatives.
4. *TMB Reagent (11 ml/bottle)*
   Contains one-step TMB solution.
5. *Stop Solution (11 ml/bottle)*
   Contains diluted hydrochloric acid (1N HCl).

MATERIALS REQUIRED BUT NOT PROVIDED
1. Distilled or deionized water
2. Precision pipettes: 5 µl, 10 µl, 50 µl, 100 µl and 1.0 ml
3. Disposable pipette tips
4. Microtiter well reader capable of reading absorbance at 450 nm.
5. Vortex mixer, or equivalent
6. Absorbent paper
7. Graph paper
8. Cardiac Marker Tri-level Control; Cat. No. 685 (Bio-Rad Laboratories Diagnostics Group, Hercules, CA 94547)

STORAGE CONDITIONS
1. Store the unopened kit at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.
2. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

REAGENT PREPARATION
1. All reagents should be allowed to reach room temperature (18-25°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 21 days when stored sealed at 2-8°C.*** Discard the reconstituted Standards after 21 days. ***To assure long term (more than 21 days) 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. Samples with expected Troponin I concentrations over 100 ng/ml may be quantitated by dilution with diluent available from vender.

INSTRUMENTATION
A microtiter well reader with a bandwidth of 10 nm or less and an optical density range of 0 to 3 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

ASSAY PROCEDURE
1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µl of standards, specimens, and controls into appropriate wells.
3. Gently mix for 10 seconds.
4. Dispense 100 µl of Enzyme Conjugate Reagent into each well.
5. Thoroughly mix for 30 seconds. It is very important to mix completely.
6. Incubate at room temperature (18-25°C) for 90 minutes.
7. Remove the incubation mixture by flicking plate contents into a waste container.

8. Rinse and flick the microtiter wells 5 times with distilled or deionized water. (Please do not use tap water.)
9. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
10. Dispense 100 µl of TMB Reagent into each well. Gently mix for 10 seconds.
11. Incubate at room temperature for 20 minutes.
12. Stop the reaction by adding 100 µl of Stop Solution to each well.
13. Gently mix for 30 seconds. ***It is important to make sure that all the blue color changes to yellow color completely.***
14. Read absorbance at 450nm with a microtiter well reader ***within 15 minutes.***

CALCULATION OF RESULTS

1. Calculate the mean absorbance value (OD_{450}) 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. Samples with cTnI concentrations greater than 75 ng/ml should be diluted 10-fold with vender's Troponin I Sample Diluent. The final cTnI values should be multiplied by 10 to obtain cTnI results in ng/ml.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with absorbency readings at 450nm shown 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.

A. Example of Standard Carve:

cTnI (ng/ml)	Absorbance (450 nm)
0	0.048
2.0	0.110
7.5	0.307
30	1.357
75	2.853