Product information

User´s Manual

Cardiac Troponin I (cTnI) ELISA

Sandwich enzyme-linked immune-sorbent assay for the determination of human cTnI in serum, cell culture supernatants or tissue homogenate.

Catalog No.: BE69227

Σ 96

Storage: 2-8°C

RUO **For Research Use Only – Not for Use in Diagnostic Procedures.**

Manufactured for:
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1 INTRODUCTION

1.1 Intended Use

Sandwich enzyme-linked immune-sorbent assay (ELISA) for the determination of human cTnI in serum, cell culture supernatants or tissue homogenate. For research use only, not for use in diagnostic procedures.

1.2 Background

Troponin I is one of 3 subunits that form the troponin complex of the thin filaments of striated muscle, the others are troponin T and troponin C. Human troponin I is presented in cardiac muscle tissue by a single isoform with molecular weight 23876 Da and it consists of 209 amino acid residues. It binds actin and inhibits actomyosin ATPase activity in the absence of calcium. In 2000, Labarrere et al. found that persistent elevation of cardiac troponin I levels after heart transplantation were associated with high risk for developing coronary artery disease and graft failure after cardiac transplantation. The cardiac troponin I may be a useful tool in identifying subjects with heart failure who are at increased risk for progressive ventricular dysfunction and death.

2 PRINCIPLE OF THE TEST

This kit is based on sandwich enzyme-linked immune-sorbent assay technology. Anti-cTnI monoclonal antibody is pre-coated onto 96-well plates. The standards and test samples are added to the wells and any cTnI present is bound by the immobilized antibody. After washing away any unbound substances, the HRP conjugated anti-cTnI monoclonal antibody is added to wells as the detection antibody. Following a wash to remove any unbound antibody-enzyme reagent, the TMB substrates are added to visualize the HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding stop solution. The density of yellow is proportional to the cTnI amount of sample captured in plate. The O.D. absorbance is read at 450nm using a microplate reader, and then the concentration of cTnI can be calculated.

3 WARNINGS AND PRECAUTIONS

1. This kit is for research use only, not for use in diagnostic procedures.
2. All reagents should be considered as potentially hazardous. It is recommend that this kit is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
3. **Store HRP conjugated anti-human cTnI antibody (concentrated) (Kit Component 4) at 4°C, do NOT store it at -20°C.**
4. Do not use expired components or mix components from different lots or suppliers.
5. Avoid contact of skin or mucous membranes with kit reagents or specimens.
6. Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
7. Avoid contact of substrate solution with oxidizing agents and metal.
8. Avoid splashing or generation of aerosols.
9. To avoid microbial contamination or cross-contamination of reagents or specimens, it is recommended to use the clean and separate pipette tip for each.
10. Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent. Exposure to acid inactivates the conjugate.
11. Distilled or deionized water must be used for reagent preparation.
12. Substrate solution must be equilibrated at room temperature prior to use.

4 REAGENTS

4.1 Reagents provided

1. One 96-well plate pre-coated with anti-human cTnI antibody
2. Lyophilized human cTnI standards: 2 tubes (20 ng / tube)
3. Sample / Standard diluent buffer: 30 ml
4. HRP conjugated anti-human cTnI antibody (Concentrated): 130 µl. Dilution: 1:100 (Store at 4℃, do NOT store it at -20℃)
5. Antibody diluent buffer: 12 ml
6. TMB substrate: 10 ml
7. Stop solution: 10 ml
8. Wash buffer: 30 ml (25x). Dilution: 1:25

Note: Reconstitute standards and test samples with Kit Component 3.

4.2 Materials required but not provided

1. Microplate reader (wavelength: 450nm)
2. Precise pipette and disposable pipette tips
3. Deionized or distilled water
4. Automated plate washer
5. Beakers, flasks or cylinders, etc.
6. Statistical calculator
7. Plate cover
8. Absorbent filter papers or absorbent material
9. ELISA shaker

4.3 Storage Conditions / Expiration

Store at 4°C for 6 months, or at -20°C for one year. Store HRP conjugated antibody at 4°C for one year.

4.4 Preparation of sample and reagents

The concentrated reagents should be brought to room temperature and should be diluted before starting the test procedure. If crystals have formed in the reagents, warm them gently until they have completely dissolved.

1. Sample

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20℃ for long term. Avoid multiple freeze-thaw cycles.

1. Cell culture supernatants: Centrifuge to remove particulates, analyze immediately or aliquot and store at -20℃. Avoid multiple freeze-thaw cycles.

2. Serum: Coagulate the serum at room temperature (about 2 hours). Centrifuge at approximately 1000 × g for 20 min. Analyze the serum immediately or aliquot and store at -20℃. Avoid multiple freeze-thaw cycles.

3. Tissue homogenate: Cut samples and weight, add certain volume of 0.01M PBS, homogenize the tissue samples, then, centrifuge to collect the supernatant to analyze.

Note: 1. Coagulate blood samples completely, then, centrifuge, and avoid hemolysis and particle. Grossly hemolyzed or lipemic samples may not be suitable for measurement of human cTnI with this assay.

2. NaN_3 cannot be used as test sample preservative, since it is the inhibitor for HRP.

3. After collecting samples, analyze immediately or aliquot and store at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

2. Wash buffer

Pour entire contents (30 ml) of the Wash buffer (25x) (Kit Component 8) into a clean 750 ml graduated cylinder. Bring to final volume of 750 ml with distilled or deionized water. Mix gently to avoid foaming.

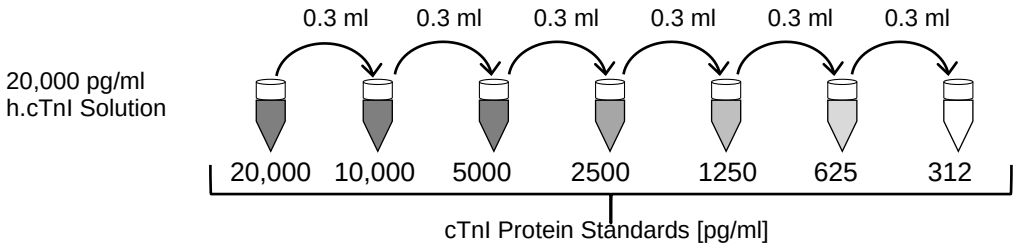
Then, transfer to a clean wash bottle and store at 2-25°C. Wash buffer (1x) can be stable for 30 days.

3. Standard

Reconstitution of the lyophilized human cTnI standard (Kit Component 2): standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of standard are included in each kit. Use one tube for each experiment. **(Note: Do not dilute the standard directly in the plate)**

a. 20,000 pg/ml of standard solution: Add **1 ml** of Sample / Standard diluent buffer (Kit Component 3) into one Standard (Kit Component 2) tube, keep the tube at room temperature for 10 min and mix thoroughly.

b. 10,000 pg/ml → 312 pg/ml of standard solutions: Label 6 Eppendorf tubes with 10,000 pg/ml, 5000 pg/ml, 2500 pg/ml, 1250 pg/ml, 625 pg/ml, 312 pg/ml, respectively. Aliquot **0.3 ml** of the Sample / Standard diluent buffer (Kit Component 3) into each tube. Add **0.3 ml** of the above 20,000 pg/ml standard solution into 1st tube and mix thoroughly. Transfer **0.3 ml** from 1st tube to 2nd tube and mix thoroughly. Transfer **0.3 ml** from 2nd tube to 3rd tube and mix thoroughly, and so on.



Note: The standard solutions are best used within 2 hours. The 20,000 pg/ml standard solution should be used within 12 hours. Or store at -20℃ for up to 48 hours. Avoid repeated freeze-thaw cycles.

4. Preparation of HRP conjugated anti-human cTnI antibody (Kit Component 4) working solution: the HRP conjugated antibody should be used within 30 min after diluting.

a. Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)

b. Dilute the HRP conjugated anti-human cTnI antibody (Kit Component 4) with Antibody diluent buffer (Kit Component 5) at 1:100 and mix thoroughly. i.e. Add 1 µl of HRP conjugated anti-human cTnI antibody into 99 µl of Antibody diluent buffer.

5 ASSAY PROCEDURE

5.1 Test Procedure

Equilibrate all kit components to room temperature before use.

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and then, record their positions. It is recommend to measure each sample, standard, zero and optional control sample in duplicate. Remove extra microwell strips from holder and store them in foil bag at 4℃, or at -20℃ for long term.
2. Aliquot 0.1 ml of 20,000 pg/ml, 10,000 pg/ml, 5000 pg/ml, 2500 pg/ml, 1250 pg/ml, 625 pg/ml, 312 pg/ml standard solutions into the standard wells.
3. Add 0.1 ml of Sample / Standard diluent buffer (Kit Component 3) into the control (zero) well.
4. Add 0.1 ml of properly diluted sample (human serum, cell culture supernatants or tissue homogenate) into test sample wells.

Here is an example of the arrangement of sample, standard and zero in the plate wells:

	1	2	3	4
A	Standard 1 (20,000 pg/ml)	Standard 1 (20,000 pg/ml)	Sample 1	Sample 1
B	Standard 2 (10,000 pg/ml)	Standard 2 (10,000 pg/ml)	Sample 2	Sample 2
C	Standard 3 (5000 pg/ml)	Standard 3 (5000 pg/ml)	Sample 3	Sample 3
D	Standard 4 (2500 pg/ml)	Standard 4 (2500 pg/ml)	Sample 4	Sample 4
E	Standard 5 (1250 pg/ml)	Standard 5 (1250 pg/ml)	Sample 5	Sample 5
F	Standard 6 (625 pg/ml)	Standard 6 (625 pg/ml)	Sample 6	Sample 6
G	Standard 7 (312 pg/ml)	Standard 7 (312 pg/ml)	Sample 7	Sample 7
H	Zero / Blank	Zero / Blank	Sample 8	Sample 8

5. Seal the plate with a cover and incubate at 37℃ for 90 min.
6. Remove the cover, aspirate the plate content and wash plate 3 times with Wash buffer (Kit Component 8) using one of the following methods:

Manual Washing: Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with Wash buffer (Kit Component 8) and vortex mildly

on ELISA shaker for 2 min, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure two more times for a **total of THREE washes**.

Automated Washing: Aspirate all wells, then wash plate **THREE times** with Wash buffer (Kit Component 8) (overfilling wells with the buffer, about 400µl). After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 min or shaking.

7. Add 0.1 ml of HRP conjugated anti-human cTnI antibody work solution into each well. Add the solution at the bottom of each well without touching the side wall.
8. Seal the plate with a new cover and incubate at 37℃ for 60 min.
9. Remove the cover, aspirate the plate content and wash plate **FIVE times** with Wash buffer (Kit Component 8), and each time let the wash buffer stay in the wells for 1-2 min. (Take reference of Step 6 for washing).
10. Add 0.1 ml of TMB substrate (Kit Component 6) into each well, cover the plate and incubate at 37℃ in dark within 30 min. (**Note:** The color development on the plate should be monitored and the substrate reaction stopped before positive wells are no longer properly recordable. Determination of the ideal time period for color development has to be done individually for each assay. It is recommended to add the stop solution when the highest standard has developed a dark blue color.) And the shades of blue can be seen in the first 3-4 wells (with most concentrated human cTnI standard solutions), the other wells show no obvious color.
11. Add 0.1 ml of Stop solution (Kit Component 7) into each well and mix thoroughly. The color changes into yellow immediately.
12. Read the O.D. absorbance at 450 nm in a microplate reader within 30 min after adding the stop solution.

Note: If the incubation without shaking, the obtained O.D. Values may be lower than the typical data, but the results are still valid.

5.2 Results

For calculation, average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. (The relative $O.D._{450}$) = (the $O.D._{450}$ of each well) – (the $O.D._{450}$ of Zero well). The standard curve can be plotted as the relative $O.D._{450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The human cTnI concentration of the samples can be interpolated from the standard curve.

Note: 1. If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

2. Calculation of samples with a concentration exceeding 10,000 pg/ml may result in incorrect, low the human cTnI levels. Such samples require further external pre-dilution according to expected human cTnI values with Sample / Standard diluent buffer (Kit Component 3) in order to precisely determine the actual human cTnI level.

6 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance. It is recommended to use controls according to state and federal regulations. The use of controls is advised to assure the day to day validity of results. It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results. Employ appropriate statistical methods for analyzing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials, results of unknowns should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. After checking the above mentioned items without finding any error contact your distributor or IBL-America directly.

7 PERFORMANCE CHARACTERISTICS

7.1 Range

312 pg/ml - 20,000 pg/ml

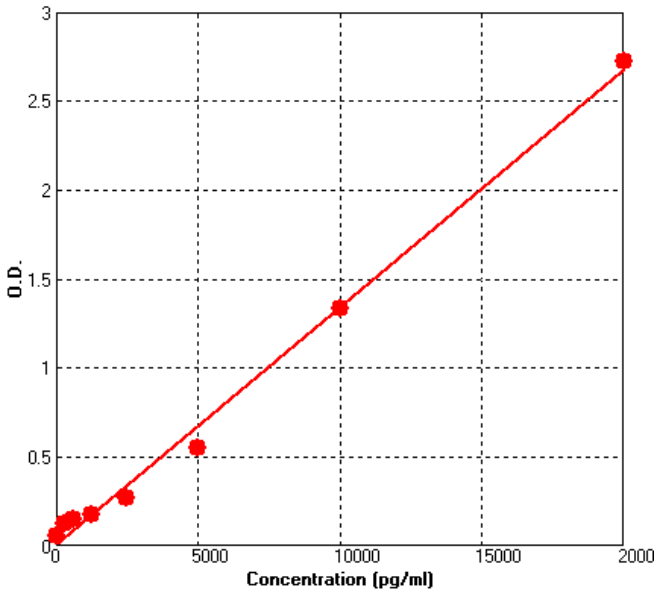
7.2 Sensitivity

10 pg/ml

7.3 Typical Data & Standard Curve

Results of a typical standard run of a human cTnI ELISA Kit are shown below. This standard curve was generated at our lab for demonstration purpose only. Each user should obtain their own standard curve as per experiment. (N/A=not applicable)

X	pg/ml	0	312	625	1250	2500	5000	10,000	20,000
Y	OD450	0.053	0.126	0.146	0.174	0.266	0.546	1.336	2.722



7.4 References:

1. Horwich, T. B., Patel, J., MacLellan, W. R., Fonarow, G. C. Cardiac troponin I is associated with impaired hemodynamics, progressive left ventricular dysfunction, and increased mortality rates in advanced heart failure. Circulation 108: 833-838, 2003.

2. Labarrere, C. A., Nelson, D. R., Cox, C. J., Pitts, D., Kirlin, P., Halbrook, H. Cardiac-specific troponin I levels and risk of coronary artery disease and graft failure following heart transplantation. JAMA 284: 457-464, 2000.

8 ORDERING INFORMATION

This kit is manufactured for Immuno-Biological Laboratories, Inc. (IBL-America). For ordering information, please contact:

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