Product Manual

Human Cardiac Troponin I (cTnI) ELISA Kit

Catalog Numbers

PRB- 5050	96 assays
PRB- 5050- 5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Troponin is an important contractile regulatory protein of skeletal and cardiac muscle. Troponin exists as a heteromeric protein complex (Figure 1) in striated muscle and consists of three subunits: troponin C (TnC), troponin T (TnT), and troponin I (TnI). Each of these subunits plays a specific regulatory function in this complex. TnC binds Ca^{2+} ions, TnT binds tropomyosin, and TnI binds actin; when complexed, they attach to the actin filament.

Troponin I exists in 3 isoforms. The TnI isoform found in the myocardium, cTnI, has been shown to be a powerful diagnostic marker for assessing heart disorders. Following a heart attack (myocardial infarction), damaged cells release cTnI into the blood; these elevated levels can be seen 3-6 hours post-infarction and remain elevated for several days. Over the last 20 years, cTnI has emerged as the preferred biomarker for myocardial infarction diagnosis and is considered more sensitive/specific than other diagnostic targets such as CK-MB, total CK, myoglobin, or LDH.

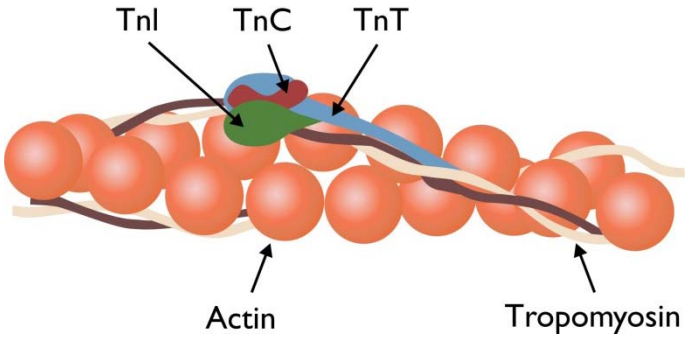

Figure 1: The Troponin Complex

Cell Biolabs' Human Cardiac Troponin I ELISA Kit is an enzyme immunoassay developed for detection and quantitation of the human cardiac Troponin I protein. The kit has a detection sensitivity limit of 50 pg/mL cTnI. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and Troponin I samples.

Assay Principle

An anti-Troponin I coating antibody is adsorbed onto a microtiter plate. Troponin I protein present in the sample or standard binds to the antibodies adsorbed on the plate; a biotinylated anti-Troponin I antibody is added and binds to the antigen captured by the first antibody. Following incubation and wash steps, a streptavidin-enzyme conjugate is added and binds to the biotinylated anti-Troponin I antibody. Unbound streptavidin-enzyme conjugate is removed during a wash step, and substrate solution is added to the wells. A colored product is formed in proportion to the amount of Troponin I present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from purified cardiac Troponin I and sample concentration is then determined.

Related Products

1. PRB-5033: Human Alpha 2 Macroglobulin ELISA Kit
2. PRB-5034: Human Alpha 1 Antitrypsin ELISA Kit
3. PRB-5038: Human Beta 2 Microglobulin ELISA Kit
4. PRB-5039: Human Haptoglobin ELISA Kit
5. PRB-5041: Human Ceruloplasmin ELISA Kit
6. PRB-5044: Human Alpha 1 Antitrypsin ELISA Kit
7. PRB-5047: Human CK-MB ELISA Kit
8. PRB-5048: Human D-Dimer ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. Anti-Troponin I Antibody Coated Plate (Part No. 50501B): One strip well 96-well plate.
2. Biotinylated Anti-Troponin I Antibody (1000X) (Part No. 50502D): One 20 µL vial.
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Human Cardiac Troponin I Standard (Part No. 50503D): One 100 µL vial of 2 µg/mL human cardiac Troponin I.

Materials Not Supplied

1. Cardiac Troponin I Sample: serum, plasma, lysate
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receiving, aliquot and store Troponin I Standard at -20ºC and avoid freeze/thaw. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-Troponin I Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-Troponin I Antibody 1:1000 and the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

1. Prepare a dilution series of Troponin I Standard in the concentration range of 2 ng/mL – 0.031 ng/mL by diluting the stock solution in Assay Diluent (Table 1).

Standard Tubes	2 µg/mL Human Cardiac Troponin I Standard (µL)	Assay Diluent (µL)	cTnI (ng/mL)
1	4	3996	2
2	500 of Tube #1	500	1
3	500 of Tube #2	500	0.5
4	500 of Tube #3	500	0.25
5	500 of Tube #4	500	0.125
6	500 of Tube #5	500	0.063
7	500 of Tube #6	500	0.031
8	0	500	0

Table 1. Preparation of Troponin I Standard

Assay Protocol

1. Prepare and mix all reagents thoroughly before use.
2. Add 100 µL of Troponin I sample or standard to the Anti-Troponin I Antibody Coated Plate. Each Troponin I sample, standard, blank, and control should be assayed in duplicate.
3. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
4. Remove plate cover and empty wells. Wash microwell strips 5 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
5. Add 100 µL of the diluted Biotinylated Anti-Troponin I Antibody to each well.
6. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
7. Remove plate cover and empty wells. Wash the strip wells 5 times according to step 4 above.
8. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well.
9. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.

10. Remove plate cover and empty wells. Wash microwell strips 5 times according to step 4 above. Proceed immediately to the next step.
11. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 5-20 minutes.

    Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
12. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
13. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical Troponin I ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

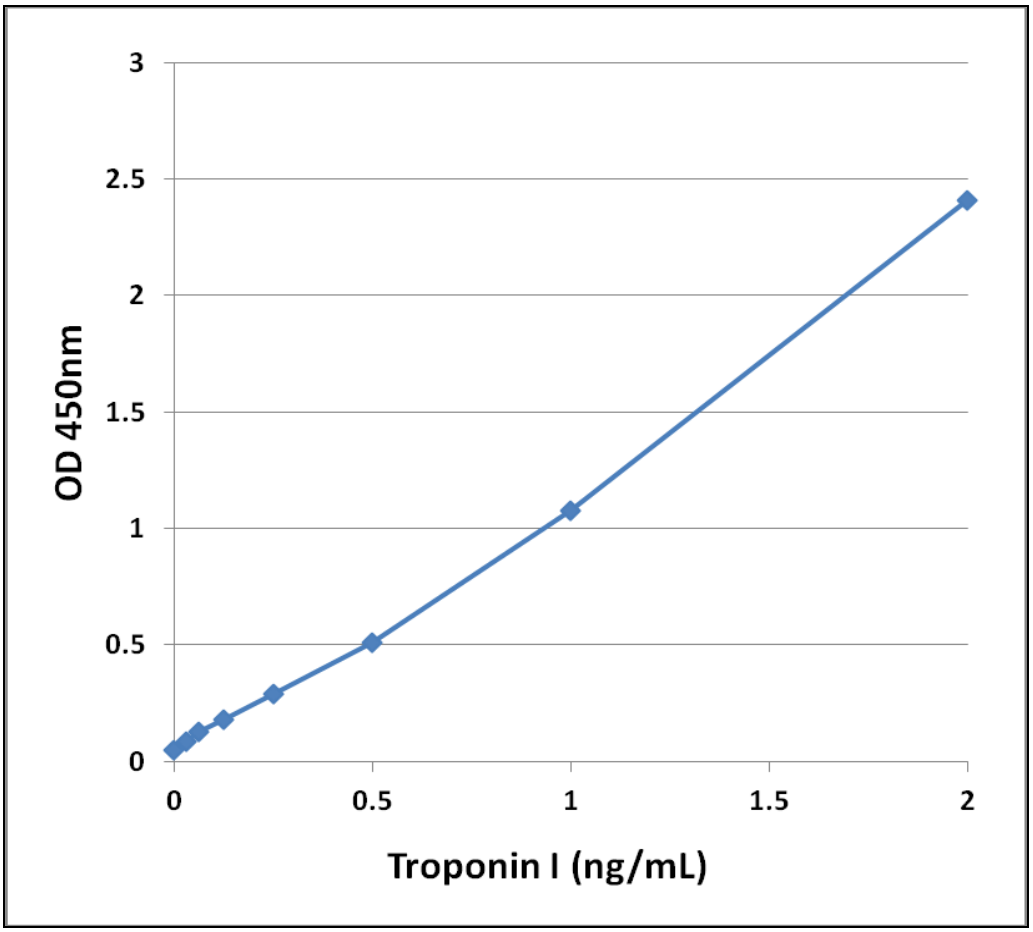

Figure 2: Troponin I ELISA Standard Curve

References

1. Corin, S. et al. (1994) *J. Bio. Chem*. **269**: 10651-7.
2. Mair, J. et al. (1993) *The Lancet* **341**: 838-9.
3. William, J., and R. Grand (1978) *Nature* **271**: 31-35.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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