



Human Troponin I ELISA

Cat. No.: RLF-EK0128R

1. Introduction

Troponin complex consists of three components troponin I, T and C. The complex along with tropomyosin is located on the actin filament and is essential for the calcium-mediated regulation of skeletal and cardiac muscle contraction. Three isoforms of troponin I have been described for striated muscle. Two isoforms are characteristic for fast and slow skeletal fibers and one isoform for cardiac muscle.

Troponin I consists of 181-211 amino acid residues, and the cardiac isoform is larger due to the presence of an additional N-terminal peptide which has a biologically important function in the interactions of troponin I and troponin C. The main function of troponin I is the inhibition of actomyosin ATPase activity.

Cardiac troponin I and T are sensitive biomarkers of myocardial injury and have become central to the diagnosis of myocardial infarction. They are elevated in many clinical syndromes associated with direct myocardial injury, myocardial ischemia, or ventricular strain. They are also released in a number of clinical situations in which thrombotic complications of coronary artery disease. These situations include conditions like pulmonary embolism, sepsis, myocarditis, and acute stroke.

2. Principles of Method

The design of this assay is based on a sandwich Enzyme-Linked Immunosorbent Assay (ELISA). The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to human Troponin I. Samples are pipetted into these wells. Unbound Troponin I and other components of the sample are removed by washing, then biotin-conjugated monoclonal antibody specific to Troponin I is added. In order to quantitatively determine the amount of Troponin I present in the sample, Avidin conjugated to Horseradish Peroxidase (HRP) is then added to each microplate well. Next, a TMB-substrate solution is added to each well. Finally, a sulfuric acid solution is added and the resulting yellow colored product is measured at 450 nm. The absorbance (O.D. value) is directly proportional to the amount of captured Troponin I.

3. Intended Use

The AbFrontier human Troponin I ELISA kit is to be used for the in vitro quantitative determination of human Troponin I in human serum, human plasma, buffered solution, or cell lysate.

This kit has been configured for research use only and is not to be used in diagnostic procedures.

4. Storage and Stability

All components of this kit are stable at 2 to 8°C. Any unused reconstituted standard should be discarded or frozen at -70°C. Standard can be frozen and thawed one time only without loss of immunoreactivity.

5. Chemical Hazard

- Stop solution: This reagent is an irritant to eyes, skin and mucous membranes. Avoid contact with eyes, skin and clothing. Wear suitable protective clothing, gloves and eye protection. In the event of contact with eyes or skin, wash immediately with plenty of water.
- All reagents containing Sodium Azide also contain Thimerosal as a preservative. Thimerosal contains Hg thus should be handled with great care.

6. Kit Contents

Contents	Number	Volume
96 Well Plate	1 (in aluminum foil bag with desiccant)	
Incubation Buffer	1	30 ml
Washing Buffer	1	(20X) 25 ml
Standard Protein	1 Glass vial (lyophilized)	
Standard/Sample Dilution Buffer	1	25 ml
Secondary Antibody	1 Glass vial (lyophilized)	
AV-HRP	1	(100X) 150 µl
Secondary Antibody/AV-HRP Dilution Buffer	1	25 ml
Substrate (TMB)	1	15 ml
Stop Solution	1	15 ml
Protocol booklet	1	
Plate sealers	2	

① 96 Well Plate

: Human Troponin I microtiter plate, one plate of 96 wells (16well strip x 6).

A plate using break-apart strips coated with a mouse monoclonal antibody specific to human Troponin I.

② Standard Protein

: Native human Troponin I (purified from human).

③ Secondary Antibody

: Biotin labeled mouse anti human Troponin I monoclonal antibody.

④ AV-HRP

: Avidin linked Horseradish Peroxidase (HRP, enzyme)

⑤ Substrate (Stabilized chromogen)

: Tetramethylbenzidine(TMB) solution

⑥ Stop Solution

: 1N solution of sulfuric acid (H₂SO₄).

⑦ Plate sealer

: Adhesive sheet.

- Do not mix or interchange reagents from different lots.

7. Materials Required But Not Provided

- ① Microtiter plate reader capable of measurement at or near 450 nm.
- ② Calibrated, adjustable precision pipettes, preferably with disposable plastic tips
(A manifold multi-channel pipette is desirable for large assays.)
- ③ Distilled or deionized water
- ④ Data analysis and graphing software
- ⑤ Vortex mixer
- ⑥ Polypropylene tubes for diluting and aliquoting standard
- ⑦ Absorbent paper towels
- ⑧ Calibrated beakers and graduated cylinders of various sizes

8. Reagent Preparation

1) Human Troponin I standard

First, make the working standard protein solution by mixing *Standard/Sample Dilution Buffer* 1ml with *Standard Protein Stock Solution* 1 µl, which will be 1 µg/ml.

Standard	Add	Into
25.00 ng/ml	25 µl of the 1 µg/ml working std. solution	975 µl of the <i>Standard/Sample Dilution Buffer</i>
12.50 ng/ml	12.5 µl of the 1 µg/ml working std. solution	987.5 µl of the <i>Standard/Sample Dilution Buffer</i>
6.25 ng/ml	6.25 µl of the 1 µg/ml working std. solution	993.75 µl of the <i>Standard/Sample Dilution Buffer</i>
3.13 ng/ml	3.13 µl of the 1 µg/ml working std. solution	996.87 µl of the <i>Standard/Sample Dilution Buffer</i>
1.56 ng/ml	1.56 µl of the 1 µg/ml working std. solution	998.44 µl of the <i>Standard/Sample Dilution Buffer</i>
0.78 ng/ml	0.78 µl of the 1 µg/ml working std. solution	999.22 µl of the <i>Standard/Sample Dilution Buffer</i>
0.39 ng/ml	0.39 µl of the 1 µg/ml working std. solution	999.61 µl of the <i>Standard/Sample Dilution Buffer</i>
0 ng/ml	1.0 ml of the <i>Standard/Sample Dilution Buffer</i>	

2) Secondary Antibody

100X secondary antibody solution can be made by adding 150 µl secondary antibody/AV-HRP dilution buffer in the vial.

1. Equilibrate to room temperature, mix gently.
2. Mix 20ul *Secondary Antibody concentrated solution* (100X) + 2ml *Secondary Antibody/AV-HRP dilution buffer*.
(Sufficient for one 16-well strip, prepare more if necessary) Label as "Working Secondary antibody Solution".
3. Return the unused *Secondary Antibody concentrated solution* to the refrigerator.

3) AV-HRP

1. Equilibrate to room temperature, mix gently.
2. Mix 20 µl *AV-HRP concentrated solution* (100X) + 2 ml *Secondary Antibody/AV-HRP dilution buffer*. (Sufficient for one 16-well strip, prepare more as needed) Label as "Working AV-HRP Solution".
3. Return the unused *AV-HRP concentrated solution* to the refrigerator.

4) Washing buffer

1. Equilibrate to room temperature, mix to re-dissolve any precipitated salt.
2. Mix 1 volume *Wash buffer concentrate solution* (10X) + 9 volumes of deionized water. Label as "Working Washing Solution".
3. Store both the concentrated and the Working Washing Solution in the refrigerator.

* Directions for washing

1. Fill the wells with 300µl of "Working Washing Buffer".

Let soak for 1 to 3 minutes and then all residual wash-liquid must be drained from the wells by aspiration (taking care not to scratch the inside of the well) or decantation, followed by forceful tapping of the plate on absorbent paper. Never insert absorbent paper directly into the wells.

If using an automated washer, the operating instructions for washing equipment should be carefully followed.

2. Incomplete washing or residual washing buffer in wells will adversely affect the assay and render false results.
3. It is recommended to use laboratory tape to hold plate strips to the plate frame while performing the plate washing to avoid strips coming free of the frame.

9. Assay Procedure

- Allow all reagents to reach room temperature before use.
Gently mix all liquid reagents prior to use.
- All standards, controls and samples should be run in duplicate for confirmation of reproducibility.
- A standard curve must be run with each assay.
- If particulate matter is present in the analyte, centrifuge or filter prior to analysis.
- Maintain a consistent order of sample and reagent additions from well to well.
This ensures equal incubation times for all wells.

- 1) Determine the number of 16-well strips needed for assay. Insert these in the frame(s) for current use (Re-bag extra strips and frame. Refrigerate for further use).
- 2) Add 300 μ l of *Incubation buffer* to all wells and incubate the plate for 5 minutes at room temperature.
- 3) Thoroughly aspirate or decant the solution from the wells. Wash wells 2 times (See "Directions for washing").
- 3) For the standard curve, add 100 μ l of the standard to the appropriate microtiter wells. Add 100 μ l of the *Standard/Sample Dilution Buffer* to zero wells.
- 4) Serum and plasma require at least 20 fold dilution in the *Standard/Sample Dilution Buffer*. And add 100 μ l of diluted samples to each well.
- 5) Cover the plate with the plate cover and incubate for 2 hours at room temperature.
- 6) Thoroughly aspirate or decant the solution from the wells. Wash the wells 3 times (See "Directions for washing").
- 7) Pipette 100 μ l of "Working Secondary Antibody Solution" into each well.
- 8) Cover the plate with the plate cover and incubate for 1 hour at room temperature.
- 9) Thoroughly aspirate or decant the solution from the wells. Wash the wells 3 times (See "Directions for washing").
- 10) Add 100 μ l "Working AV-HRP Solution" to each well.
- 11) Cover the plate with the plate cover and incubate for 30 minutes at room temperature.
- 12) Thoroughly aspirate or decant the solution from the wells. Wash the wells 3 times (See "Directions for washing").
- 13) Pour enough Substrate that you need into a tube or reagent boat. Add 100 μ l of *Substrate* to each well. The liquid in the wells should begin to turn blue.
- 14) Incubate the plate at room temperature. Avoid exposing plate to direct sunlight.
 - Do not cover the plate with aluminum foil (or other metal), or color may develop.

The incubation time for chromogen substrate is often determined by the microtiter plate reader used. O.D. values should be monitored and the substrate reaction stopped before O.D. of the positive wells exceeds the limits of the instrument. O.D. values at 450 nm can only be read after the Stop Solution has been added to each well.

 - Keep the plate away from sunlight because the Substrate is light sensitive..
 - Typically, reaction is stopped 5~10 minutes after treatment of Substrate, but this time can be adjusted as the user desires.
- 15) Add 100 μ l of *Stop Solution* to each well. The solution in the wells should change from blue to yellow.
- 16) Read the absorbance of each well at 450nm. Read the plate within 20 minutes of adding the *StopSolution*.

- 17) Plot on graph paper the absorbance of the standard against the standard concentration (Optimally, the background absorbance can be subtracted from all data points, including standards, unknowns and controls, prior to plotting.). Draw a smooth curve through these points to construct the standard curve.
- 18) Read the human Troponin I concentrations for the unknown samples and controls from the standard curve plotted in step 18. Multiply value(s) obtained for the unknown sample by the dilution factor (Samples producing signals greater than that of the highest standard should be further diluted in the *Standard/Sample Dilution Buffer*).

10. Characteristics

1) Typical result

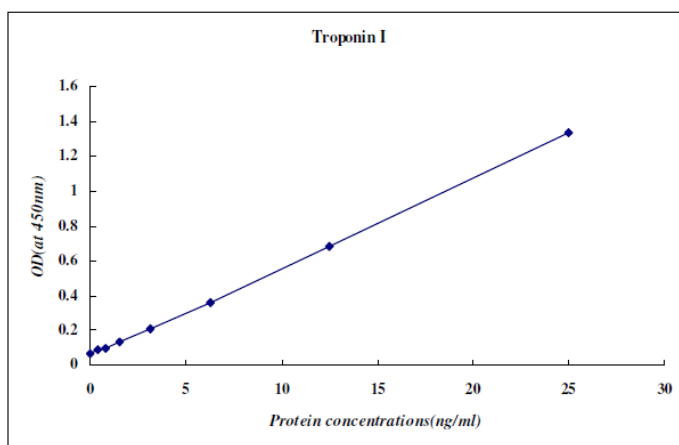
The standard curve below is for illustration only and **should not be used** to calculate results in your assay.

A standard curve must be run with each assay.

Standard Human Troponin I (ng/ml)	Optical Density (at 450nm)
0	0.070
0.39	0.090
0.78	0.100
1.56	0.134
3.13	0.212
6.25	0.362
12.50	0.680
25.00	1.337

Limitations

- Do not extrapolate the standard curve beyond the 25 ng/ml standard point.
- Other buffers and matrices have not been investigated.
- The rate of degradation of native human Troponin I in various matrices has not been investigated.



2) Sensitivity

The minimal detectable dose of human Troponin I was calculated to be 0.38 ng/ml, by subtracting two standard deviations from the mean of 10 zero standard replicates (ELISA buffer, S0) and intersecting this value with the standard curve obtained in the same calculation.

3) Specificity

The following substances were tested and found to have no cross-reactivity: cardiac Troponin T and cardiac Troponin C. Muscle Troponin I had slight cross-reactivity.

4) Precision

① Within-Run (Intra-Assay)

(n=6)

Mean (ng/ml)	SD (ng/ml)	CV (%)
3.11	0.19	6.0
6.02	0.35	5.9
12.28	1.09	8.9
23.15	0.85	3.7

② Between-Run (Inter-Assay)

(n= 3)

Mean (ng/ml)	SD (ng/ml)	CV (%)
3.01	0.01	0.4
5.83	0.08	1.4
11.32	0.37	3.3
25.6	1.57	6.1

5) Recovery

Recovery upon addition is 98.3~104.2% (mean 100.8%)

Recovery upon dilution is 99.3~103.9% (mean 100.7%)

11. Troubleshooting

Problem	Possible Cause	Solution
High signal and background in all wells	• Insufficient washing	• Increase number of washes • Increase time of soaking between in wash
	• Too much AV-HRP	• Check dilution, titration
	• Incubation time too long	• Reduce incubation time
	• Development time too long	• Decrease the incubation time before the stop solution is added
No signal	• Reagent added in incorrect order, or incorrectly prepared	• Review protocol
	• Standard has gone bad (If there is a signal in the sample wells)	• Check the condition of stored standard
	• Assay was conducted from an incorrect starting point	• Reagents allows to come to 20~30°C before performing assay
Too much signal – whole plate turned uniformly blue	• Insufficient washing – unbound AV-HRP remaining	• Increase number of washes carefully
	• Too much AV-HRP	• Check dilution
	• Plate sealer or reservoir reused, resulting in presence of residual AV-HRP	• Use fresh plate sealer and reagent reservoir for each step
Standard curve achieved but poor discrimination between point	• Plate not developed long enough	• Increase substrate solution incubation time
	• Improper calculation of standard curve dilution	• Check dilution, make new standard curve
No signal when a signal is expected, but standard curve looks fine	• Sample matrix is masking detection	• More diluted sample recommended
Samples are reading too high, but standard curve is fine	• Samples contain protein levels above assay range	• Dilute samples and run again
Edge effect	• Uneven temperature around work surface	• Avoid incubating plate in areas where environmental conditions vary • Use plate sealer

12. References

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- Babuín L and Jaffe AS. Troponin: the biomarker of choice for the detection of cardiac injury. 2005, CMAJ. 173(10):1191-1202
- Hunkeler NM et al. Troponin I isoform expression in human heart. 1991, Circ. Res. 69;1409-1414

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