cTNI (Troponin I) ELISA

CAT NO	DESCRIPTION	PACK SIZE
EIATNI1	Troponin I ELISA	96 Tests

Intended Use:

Troponin I ELISA is intended for the quantitative determination of Cardiac-Specific Troponin I concentrations in human serum. This reagent is for In vitro Diagnostic use only.

Summary and Principle:

Cardiac Troponin is the regulatory subunit of the Troponin Complex, it regulates the calcium modulated interaction of actin and myosin in striated muscle. The complex is a heterodimer consisting of troponins C, I and T, which are tightly bound to the contractile apparatus; hence the circulating levels are low. Recent clinical studies have shown that the dissociation of the troponin complex following myocardial damage and the release of individual protein components into the bloodstream. Cardiac Troponin I is a marker of choice of heart damage and myocardial cell death.

The Cardiac-Specific Troponin I ELISA test is a quantitative enzyme immunoassay that provides a rapid, sensitive and reliable assay for the quantitative measurement of cardiac -specific troponin I. The test kit can be used together with other diagnostics method to assess cardiac damage caused by AMI.

This assay is based on the principle of a solid phase enzyme linked immunosorbent assay. The assay utilises microtiter wells coated with monoclonal anti-TnI antibody. Other monoclonal anti-TnI antibody is contained in the antibody-horseradish peroxidase (HRP) conjugate solution. The test sample is allowed to react simultaneously with the antibodies and the troponin I molecules are sandwiched between the immobilised and enzyme-linked antibodies. After a 15minute incubation, the wells are washed to remove unbound labelled antibodies and a TMB substrate solution is added. The reaction between HRP and the substrate results in the development of a blue colour which is stopped with the addition of 2N hydrochloric acid changing the solution to yellow. The concentration of troponin I is directly proportional to the colour which is measured spectrophotometrically at 450nm.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Antibody coated-micotiter wells	1x96 wells	Each microwell is coated with monoclonal anti-TnI antibody. Place unused wells or strips in the provided plastic sealable bag together with the desiccant and store at 2-8°C.
Troponin I Calibrators (lyophilised)	6x0.5ml	6 vials containing Troponin I, traceable to NIST Troponin I 2921. THE EXACT CONCENTRATIONS ARE PROVIDED ON THE VIAL LABEL. CONCENTRATIONS GIVEN IN THE IFU ARE SUBJECT TO CHANGE. ***Reconstitute by addition of 0.5ml of distilled water and allow to stand for 20 minutes***. Once open stable for 24 hours at 2-8°C. For longer storage aliquot and freeze at -20°C. DO NOT FREEZE THAW MORE THAN ONCE.
Control Level 1 (Lyophilised)	1x0.5ml	1 vial containing Troponin I. ***Reconstitute by addition of 0.5ml of distilled water and allow to stand for 20 minutes***. Once open stable for 24 hours at 2-8°C. For longer storage aliquot and freeze at -20°C. DO NOT FREEZE THAW MORE THAN ONCE.
Control Level 2 (Lyophilised)	1x0.5ml	1 vial containing Troponin I. ***Reconstitute by addition of 0.5ml of distilled water and allow to stand for 20 minutes***. Once open stable for 24 hours at 2-8°C. For longer storage aliquot and freeze at -20°C. DO NOT FREEZE THAW MORE THAN ONCE.
Enzyme Conjugate	1x12ml	1 vial containing 12ml of HRP labelled monoclonal anti-TnI antibody in Buffered saline. Store at 2-8°C.
Wash Buffer Concentrate	1x15ml	PBS-Tween at pH 7.4. 50X concentrate. Once open, stable for one month at 2-8°C. The concentrate must be diluted to 750ml with distilled water. Once diluted it is stable between 2 – 30°C for two months.
TMB Substrate	1x12ml	TMB in buffer. Ready to use. Store at 2-8°C.
Stop Solution	1x12ml	Diluted HCl (2N) Ready to use. Once open, stable for 2 months at 2-30°C.

Plastic Sealable bag, IFU and Cardboard plate covers.

Materials required but not provided:

Distilled water, Vortex mixer, Micropipettes and Disposable tips, Incubator, Microplate Reader and Microplate washer, QC Materials, Absorbent paper.

Specimen Collection:

Serum is the sample of choice. Collect serum samples in accordance with correct medical practices. Ensure that the samples are clear and do not have suspended particles or sediments. Avoid usage of highly lipaemic, haemolytic or turbid samples. Assay samples immediately. Store at 2-8°C and assay within 5 days. Store at -20°C and assay within 30 days. Avoid multiple freeze thaw cycles. After thawing, bring to room temperature and mix well by gentle shaking.

Storage and Stability:

The contents of the kit will remain stable up to expiry date when stored at 2-8°C. Do not freeze. Keep all components tightly capped and without any contamination. Place unused wells in zip-lock bag provided and return to 2-8°C, under which conditions the wells will remain stable for 2 months, or until the labelled expiry date, whichever is earlier. Seal and return all the other unused reagents to 2-8°C, under which conditions the stability will be retained for 2 months, or until the labelled expiry date, whichever is earlier.

Precautions and Safety:

ELISAs are time and temperature sensitive. To avoid incorrect results, strictly follow the test procedure and do not modify them.

1. For professional use only.
2. Follow the instructions in this IFU as reliability of results cannot be guaranteed if there are deviations from the instructions.
3. The calibrators contain human serum based components. They have been tested and found to be non-reactive to HBsAg, HIV and HCV antibodies and syphilis. The assay contains materials of animal origin like BSA which have been sourced from countries where BSE has not been reported. It is recommended that all human serum based material may be considered potentially infectious and care to be taken in their use.
4. Wear laboratory protection equipment such as gloves, glasses whilst handling reagents, controls and samples. Wash hands thoroughly after each operation.
5. Samples in the microwells should not have bubbles as these bubbles may result in erroneous results.
6. Wash the wells completely. Avoid overflow during wash. Remove any residual wash buffer by tapping the microwells on absorbent paper. It is ideal to use an automated microplate washer.
7. Use new pipette tips for each pipetting to avoid cross contamination.
8. Do not use kits after expiry date
9. Do not interchange components from other kits.
10. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore the substrate and stop solution should be added in the same sequence to eliminate any time deviation during reaction.
11. If more than one plate is used, it is recommended that the calibration curve is repeated.
12. Secure the calibrator vial caps, if unused calibrators are stored for further use.
13. It is important that the time of reaction in each well is held constant to achieve reproducible results.
14. It is important to calibrate all equipment e.g. micropipettes, microplate readers, automated microplate strip washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
15. Ensure that the bottom of the plate is clean and dry and that no bubbles are present on the surface of the liquid before reading the plate.
16. Failure to remove adhering solution adequately in the washing step will lead to erroneous results.
17. Use no more than 32 wells for each assay run, when manual pipette is used. Complete pipetting of all calibrators, controls and samples within 5 minutes.
18. DO NOT USE REAGENTS THAT ARE CONTAMINATED.

Procedure:

Reagent preparation:

1. Bring all reagents to room temperature prior to use.
2. **Wash Buffer**: Make up the wash buffer solution to 750ml using distilled water. This diluted wash buffer is stable for 2 months at 2-30°C.

STEP 1

Preparation: Remove the number of wells required and number each well for the assay series.

STEP 2

Addition of Samples and calibrators: Add 25ul of Calibrators, Controls and Samples to each well.

STEP 3

Addition of Enzyme Conjugate: Add 100ul of the Enzyme Conjugate solution to each well. Shake the plate for 30 seconds to ensure that the added components are well mixed. Ensure that the conjugate is dispensed close to the bottom of the wells. Use new tips for every well. Use a multichannel pipette to quickly dispense the Enzyme reagent to avoid drift if the dispensing is to take more than a few minutes.

STEP 4

Incubation: Cover the plate with the plate cover and incubate for 15 minutes at room temperature.

STEP 5

Washing: At the end of the incubation period, remove the plate cover and discard the well contents to waste. Wash each well 3 times with 350ul of diluted wash buffer. After the final washing cycle, turn down the plate onto absorbent paper and tap it to remove any residual buffer.

STEP 6

Addition of the Substrate: Add 100ul of TMB solution to each well. Gently mix for 5 seconds.

STEP 7

Incubation: Cover the plate with the plate cover and incubate for 15 minutes at room temperature. Ensure that the incubation is done in the dark.

STEP 8

Stopping the Reaction: Add 100ul of the Stop solution into each well and mix gently. Shake the plate to mix till the solution changes to from blue to yellow.

STEP 9

Measurement: Read the absorbance of the wells at 450/630nm using a microplate reader. Note down the absorbances.

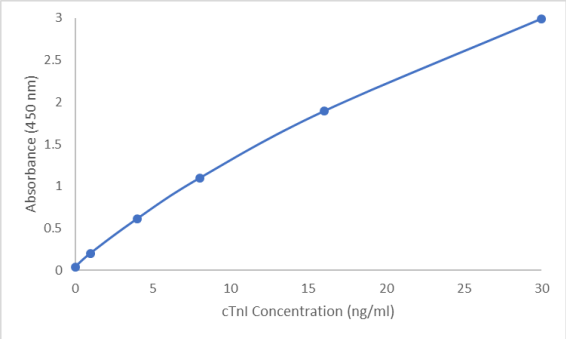
Calculation of results:

- Record the absorbances obtained from the microplate reader. Ensure that mean absorbances are calculated for duplicate measurements.
- Plot the absorbance in Y axis and Concentration in ng/ml in X axis.
- Draw a point to point (best fit) curve through the plotted points on a linear graph paper.

- To determine the concentration of an unknown sample, locate the absorbance of the sample on the Y axis and find the intersecting point on the curve. Read the concentration from the X axis by dropping a line from the intersecting point of the absorbance on the curve.

Example:

ID	ABSORBANCE OF CALIBRATORS (450nm)	CONCENTRATION OF CALIBRATORS (ng/mL)
CAL A	0.039	0.0
CAL B	0.203	1.0
CAL C	0.613	4.0
CAL D	1.094	8.0
CAL E	1.890	16.0
CAL F	2.990	30.0



Performance Characteristics:

1. Intra assay Precision:

Five human serum panels were run in replicates of 10. Following are the results:

Panel	Replicates	Mean (ng/mL)	SD	CV%
1	10	1.18	0.039	3.27
2	10	2.13	0.043	2.02
3	10	4.47	0.16	3.58
4	10	9.28	0.624	6.78
5	10	21.3	0.734	3.45

2. Inter assay Precision:

Three human serum panels in replicates of 5 across 10 days. Following are the results:

Panel	Replicates	Mean (ng/mL)	SD	CV%
1	5	0.714	0.074	10.4
2	5	4.86	0.504	10.4
3	5	18.8	2.07	11

3. Sensitivity:

The sensitivity was determined by the variability of the 0 ng/ml serum calibrator and using the 2SD statistic to calculate the minimum dose. The assay sensitivity was found to be 0.04 ng/ml.

4. Cross Reactivity:

The cross reactivity of this assay against the substances listed below provided the following results:

Interferent	Cross reactivity
Haemoglobin	200 mg/ml
Human cardiac Troponin T	2500 ng/ml
Biotin	200 ng/ml
Bilirubin	1 mg/ml

5. Correlation:

Correlation studies were undertaken using a commercially available predicate assay. The data is summarized below:

Method	Mean	Linear Square Regression analysis	Correlation Coefficient
This method	25.3	Y=2.7361+1.1592(x)	0.9296
Reference method	24.3		

6. **High Dose Hook Effect:**

It has been demonstrated at Troponin I levels up to 20,000 ng/ml will produce a concentration measurement above 30 ng/mL which is the upper limit of linear range. However, in view of the limitations in the optical measurements it is recommended that sample dilutions be made so that accurate troponin I concentrations can be determined. A dilution of 1:10 is recommended before further analysis.

Reference range:

It is recommended that each laboratory establish its own normal reference ranges for the population that it serves.

Reference range for Adults: </= 1.5 ng/ml

Notes: RISK ANALYSIS & INTERPRETATION

- The results should not be used as the sole basis for clinical diagnosis, but be used alongside additional clinical findings to confirm the results.
- It is important that the time of reaction in each well is held constant to achieve reproducible results.
- Highly lipaemic and grossly haemolytic samples are not suitable with this assay.
- Patient samples with Troponin I concentrations above 30 ng/ml should be diluted with the zero calibrator or troponin I free pooled human serum or urine and re-assayed. Multiply the value obtained by the dilution factor to obtain the corrected value.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Measurement and interpretation of results must be performed by a skilled individual or trained professional.
- It is important to calibrate all the equipment e.g., Pipettes, Readers, Washers and /or the automated instruments used with this device and to perform routine preventive maintenance.
- Further details on Risk analysis can be requested from our technical department.
- All applicable national standards, regulations and laws including but not limited to good laboratory procedures must be strictly followed to ensure compliance and proper device usage.
- For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
- Accurate and precise pipetting as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this IFU may yield inaccurate results.
- If test kits are altered such as mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, AMS UK shall have no liability.
- If computer controlled data reduction is used to interpret the result of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
- The reagents have been formulated to eliminate maximal interference, however potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. For diagnostic purposes the results from this assay should be used in combination with clinical examination, patient's history and all other clinical findings.

References:

1. Apple Fred S, Christenson RH, Valdes RJ, Andriak AB, Duh Show-Hong, Feng YJ, Saeed AJ, Johnson Nancy J, Koplen Brenda, Mascotti K and Wu Alan J. 'Simultaneous Rapid measurement of Cardiac Troponin I by the Triage Cardiac panel for detection of Myocardial Infarction', Clin Chem 48, 199-205 (1999).
2. Adams JE, Schechtman KB, Landt Y et al, 'Comparable detection of acture myocardial infarction by CK MB isoenzyme and cardiac troponin I', Clin chem 40, 1291-5 (1994).
3. Panteghini M 'Creatinine Kinase MB isoforms', J Clin Immunoassa, 17, 30-4 (1994)
4. Lang H, Wuerzburg U, 'Creatinine kinase an enzyme of many forms', Clin chem, 28, 1439-47 (1982).

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

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