Prestige Diagnostics
Clinical Diagnostics & Point of Care Products

# cTNI (Troponin I) Elisa

| CAT NO | DESCRIPTION | PACK SIZE |
|---|---|---|
| EIATNI1 | Troponin I Elisa | 96 Tests |

## Intended Use:

Troponin I Elisa assay is intended for the quantitative determination of Circulating Troponin I concentrations in Human serum. This reagent is for In vitro Diagnostic use only.

## Summary and Principle:

Cardiac Troponin I is the inhibitory subunit of the Troponin Complex, which 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. Cardiac Troponin I is a marker of choice of heart damage and myocardial cell death.

In this assay, Troponin I calibrator, patient's sample or control specimen is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labelled antibodies (directed against distinct and different epitopes are added and the reactants mixed. Reaction between the various Troponin I antibodies and native troponin I forms a sandwich complex that binds with the streptavidin coated to the well. The employment of several serum references of known Troponin I concentrations permits the construction of a calibration curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with the Troponin I concentration.

## Reagent Composition:

| COMPONENT | SIZE | DESCRIPTION |
|---|---|---|
| Streptavidin Plate | 1x96 wells | Each microwell is coated with Streptavidin. 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) | 6x1ml | 6 vials containing Troponin I at concentrations of 0.0, 1.0, 3.0, 6.0, 15 and 30 mg/ml made up in a human serum matrix. Traceable to NIST TnI 2921. THE EXACT CONCENTRATIONS ARE PROVIDED ON THE VIAL LABEL. CONCENTRATIONS GIVEN IN THE IFU ARE SUBJECT TO CHANGE. ***Reconstitute by addition of 1ml of distilled water***. 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 | 1x13ml | 1 vial containing 13ml of HRP labelled mouse monoclonal IgG antibodies and biotin labelled antibodies in Buffered saline. Store at 2-8°C. |
| Wash Buffer Concentrate | 1x20ml | PBS-Tween at pH 7.4. 50X concentrate. Once open, stable for one month at 2-8°C. The concentrate must be diluted to 1000ml with distilled water. Once diluted it is stable between 2 – 30°C for two months. |
| Substrate A | 1x7ml | TMB in buffer. Ready to use. Store at 2-8°C. |
| Substrate B | 1x7ml | Hydrogen Peroxide in buffer. Ready to use. Store at 2-8°C. |
| Stop Solution | 1x8ml | Diluted HCl(1N) 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, 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:**

The Elisa assays 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 a clean towel or 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. Adjust the incubator to 37°C.
3. **Wash Buffer**: Make up the wash buffer solution to 1000ml using distilled water. This diluted wash buffer is not stable for 2 months at 2-30°C.
4. **Working Substrate Solution**: Pour the contents of Substrate A into the Substrate B and swirl gently. Ensure that the solution is homogeneous before use. Stable for 60 days when stored at 2-8°C, without contamination. DO NOT USE THE SUBSTRATE IF IT LOOKS BLUE.

**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 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 and discard the plate cover. Wash each well 3 times with diluted washing buffer of 350ul. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer.

**STEP 6**

**Addition of the Substrate**: Add 100ul of working Substrate Solution to each well. DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION.

**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 50ul of the Stop solution into each well and mix gently. Shake the plate to mix till the solution changes to yellow from blue.

**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 | CONCENTRATION OF CALIBRATORS |
|---|---|---|
| CAL A | 0.016 | 0.0 ng/ml |
| CAL B | 0.096 | 1.0 ng/ml |
| CAL C | 0.260 | 3.0 ng/ml |
| CAL D | 0.564 | 6.0 ng/ml |

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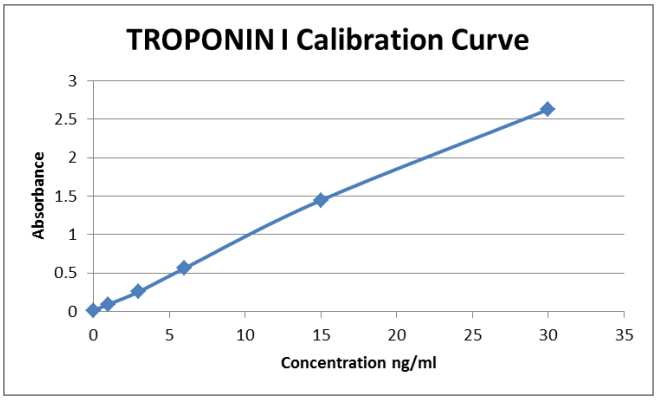
|---|---|---|
| CAL E | 1.448 | 15 ng/ml |
| CAL F | 2.830 | 30 ng/ml |
| Control Level 1 | 0.051 | 0.43 ng/ml |
| Control Level 2 | 1.245 | 12.75 ng/ml |
| Sample | 0.322 | 3.61 ng/ml |




**QC Parameters:**

1. The absorbance of Calibrator A should be </= 0.07.
2. The absorbance of Calibrator F should be >/= 1.3

**Performance Characteristics:**

1. Intra assay Precision:

Three human serum panels were run in replicates of 20. Following are the results:

| Panel | Data no. | Mean | SD | CV% |
|---|---|---|---|---|
| 1 | 20 | 0.44 | 0.014 | 3.3% |
| 2 | 20 | 3.55 | 0.072 | 2.0% |
| 3 | 20 | 12.75 | 0.311 | 2.4% |

2. Inter assay Precision:

Three human serum panels in replicates of 2 across 10 days. Following are the results:

| Panel | Data no. | Mean | SD | CV% |
|---|---|---|---|---|
| 1 | 10 | 0.48 | 0.038 | 7.9% |
| 2 | 10 | 3.68 | 0.242 | 6.6% |
| 3 | 10 | 13.58 | 0.745 | 5.5% |

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 0.030 ng/ml.

4. Analytical Specificity:

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

| Interferent | Cross reactivity |
|---|---|
| Haemoglobin | None determined |
| CK-MB | None determined |
| FABP | None determined |
| TnT | None determined |

5. Correlation:

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

| **Method** | **Mean** | **Linear Square Regression analysis** | **Correlation Coefficient** |
|---|---|---|---|
| This method | 3.04 | Y=0.3500+0.9266(x) | 0.950 |
| Reference method | 2.92 | | |

6. **High Dose Hook Effect:**

Human serum samples spiked with concentrations up to 10,000 ng/ml of cTnI did not show any hook effect with this Elisa assay.

**Reference range:**

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

**Reference range for Adults**: </= 1.3 ng/ml

**Notes: RISK ANALYSIS & INTERPRETATION**

- If the results are inconsistent with clinical findings, additional testing is suggested 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.
- 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.
- 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.
- Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy particularly if the results conflict with other determinations.
- 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.
- For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
- If test kits are altered such as mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Prestige 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:**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4. Lang H, Wuerzburg U, 'Creatinine kinase an enzyme of many forms', Clin chem, 28, 1439-47 (1982).


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| 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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