Product Information

Troponin I ELISA

Catalog Number SE120134
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Troponin is the inhibitory or contractile regulating protein complex of striated muscle. It is located periodically along the thin filament of the muscle and consists of three distinct proteins: troponin I, troponin C, and troponin T. Likewise, the troponin I subunit exists in three separate isoforms; two in fast-twitch and slow-twitch skeletal muscle fibers, and one in cardiac muscle. The cardiac isoform (cTnI) is ~40% dissimilar and has a molecular mass of 22,500 Da. Cardiac troponin I (cTnI) has been useful in the differential diagnosis of patients presenting to emergency departments (ED) with chest pain. Myocardial infarction is diagnosed when blood levels of sensitive and specific biomarkers, such as cardiac troponin, the MB isoenzyme of creatine kinase (CK-MB), and myoglobin, are increased in a setting of acute ischemia. The most recently described and preferred biomarker for myocardial damage is cardiac troponin (I or T). The cardiac troponins exhibit myocardial tissue specificity and high sensitivity. The level of cTnI remains elevated for a much longer period of time (6–10 days), thus providing for a longer window of detection of cardiac injury. Normal levels of cTnI in the blood are very low. After the onset of an acute myocardial infarction (AMI), cTnI levels increase substantially and are measurable in serum within 4–6 hours, with peak concentrations reached in 12–24 hours after infarction.

The Troponin I ELISA kit is based on the principle of a solid phase ELISA. The assay system utilizes four unique monoclonal antibodies directed against distinct antigenic determinants on the molecule. Three mouse monoclonal anti-troponin I antibodies are used for solid phase immobilization on the microplate wells. The fourth antibody is in the antibody-enzyme conjugate solution. The test sample is allowed to react simultaneously with the four antibodies, resulting in the troponin I molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 90 minute incubation at room temperature, the wells are washed with water to remove unbound-labeled antibodies. A substrate solution (TMB) is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution changing the color to yellow. The concentration of troponin I is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

Components

<table>
<thead>
<tr>
<th>Materials Provided</th>
<th>96 Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwells coated with mouse Anti-Tnl</td>
<td>12 x 8 x 1</td>
</tr>
<tr>
<td>Reference Standard Set</td>
<td>1 mL</td>
</tr>
<tr>
<td>cTnl Enzyme Conjugate Reagent</td>
<td>13 mL</td>
</tr>
<tr>
<td>TMB Reagent</td>
<td>11 mL</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>11 mL</td>
</tr>
<tr>
<td>Wash Concentrate 20x: 1 Bottle</td>
<td>25 mL</td>
</tr>
</tbody>
</table>

Reagents and Equipment Required but Not Provided.
- Distilled or deionized water
- Precision pipettes
- Disposable pipette tips
- ELISA reader capable of reading absorbance at 450 nm
- Absorbent paper or paper towel
- Graph paper
Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Sample Preparation
1. The use of serum samples is required for this test.
2. Samples should be collected using standard venipuncture techniques. Remove serum from the coagulated or packed cells within 60 minutes after collection.
3. Samples which cannot be assayed within 24 hours of collection should be frozen at –20 °C or lower, and will be stable for up to six months.
4. Avoid grossly hemolytic (bright red), lipemic (milky), or turbid samples (after centrifugation).
5. Samples should not be repeatedly frozen and thawed prior to testing. DO NOT store in "frost free" freezers, which may cause occasional thawing. Samples which have been frozen and those which are turbid and/or contain particulate matter, must be centrifuged prior to use.

Preparation of non-zero standards/calibrators
Reconstitute each lyophilized standard with 1.0 mL of distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. The reconstituted standards will be stable for up to 8 hours when stored sealed at 2–8 °C. Discard the reconstituted standards after 8 hours. To assure maximum stability of the reconstituted standards, they should be aliquoted and frozen (–20 °C or below) immediately after reconstitution has been achieved. Each aliquoted standard should be frozen and thawed only once.

20x Wash Buffer Concentrate
Prepare 1x Wash buffer by adding the contents of the bottle (25 mL, 20x) to 475 mL of distilled or deionized water. Store at room temperature (18–26 °C).

Storage/Stability
Store the kit at 2–8 °C.

Procedure
Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.

1. Secure the desired number of coated wells in holder.
2. Dispense 100 µL of standards, samples, and controls into appropriate wells.
3. Dispense 100 µL of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to mix completely.
5. Incubate at room temperature (18–25 °C) for 90 minutes.
6. Remove the incubate mixture by flicking plate contents into a waste container.
7. Remove liquid from all wells. Wash wells three times with 300 µL of 1x Wash buffer. Blot on absorbent paper or paper towel.
8. Strike the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
9. Dispense 100 µL of TMB Reagent into each well. Gently mix for 5 seconds.
10. Incubate at room temperature for 20 minutes.
11. Stop the reaction by adding 100 µL of Stop Solution to each well.
12. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
13. Read absorbance at 450 nm with a microplate reader within 15 minutes.
**Results**

**Calculations**

1. Calculate the mean absorbance value ($A_{450}$) for each set of reference standards, controls, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/mL on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of troponin I (ng/mL) from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed. The final cTnI results are in ng/mL.

**Example of typical data for a Standard Curve**

<table>
<thead>
<tr>
<th>cTnI (ng/mL)</th>
<th>Absorbance (450 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.048</td>
</tr>
<tr>
<td>2.0</td>
<td>0.110</td>
</tr>
<tr>
<td>7.5</td>
<td>0.307</td>
</tr>
<tr>
<td>30</td>
<td>1.357</td>
</tr>
<tr>
<td>75</td>
<td>2.853</td>
</tr>
</tbody>
</table>

**Expected Values**
The following values may be used as initial guideline range only. Two-hundred and twenty-five (225) apparently healthy adults were assayed using the test to establish the normal expected value, which was determined to be ≤0.5 ng/mL of cTnI. All values from the normal population tested were below the sensitivity level of the assay (1.0 ng/mL).

**Notes:** Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.

Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

**References**