In this method, Troponin-I calibrator, patient specimen or control is first reacted with a streptavidin-coated enzyme labeled antibodies (directed against and different epitopes of Troponin-I) are added and the reactants mixed. Interaction between the various Troponin-I antibodies and native Troponin-I forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-substrate complex of cTnI conjugate by conjugation or decantation. The activity of the enzyme present on the surface of the well is quantified by a reaction with a suitable substrate to produce color. The employment of several serum references of known Troponin-I concentrations to the construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen’s activity can be correlated with Troponin-I concentration.

3.0 PRINCIPLE

Sandwich Equilibrium Method (TYPE 2):

The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibody (first antibody and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay through the interaction of cTnI antibody coated on the well.

Upon mixing the enzyme-labeled antibody and a serum containing the native antigen, a reaction results between the native antigen and the immobilized antibody through the interaction of cTnI antibody coated on the well. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. cTnI Calibrators – 1.0 ml/vial (Lypophilized) – Icons [A-F]
B. cTnI Antibody Enzyme Reagent – 1 ml/vial – Icons [G-H]
C. cTnI Antibody Coat Plate – 96 wells – Icon [I]
D. Wash Solution Concentrate – 20 ml/vial – Icon [J]
E. Working Substrate Solution – 7.0 ml/vial – Icon [K]
F. Stop Solution – 8.0 ml/vial – Icon [L]
G. Substrate B – 7.0 ml/vial – Icon [S]

1. Pipette(s) capable of delivering 25µl and 100µl volumes with a precision of better than 1.5% (optional).
2. Pipette 0.025 ml (25 µl) of the appropriate calibrators, controls and patient specimen to be assayed in duplicate.
3. Add 0.050 ml (50 µl) of Troponin- I Enzyme Reagent to avoid drift if the dispensing is to take place over a long period of time.
5. Place the microplate in an incubator or water bath for 20 minutes at room temperature (20-27°C).
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350 (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) and aspirate. Repeat four (4) additional times for a total of five (5) washes. An automatic or manual plate washer can be used. Follow the manufacturer’s instructions for proper usage. If a squeeze bottle is used, fill each well to the top by squeezing the container.
8. Do not use reagents beyond the kit expiration date.
9. DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
10. Incubate at room temperature for fifteen (15) minutes.

Reagent Information:

1. Format the microplates’ wells for calibrator, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. Pipette 0.025 ml (25µl) of the appropriate calibrators, controls and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
3. Do not use reagents that are contaminated or have bacteria growth.
5. DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
6. Place the microplate in an incubator or water bath for 20 minutes at room temperature (20-27°C).
7. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section).
9. Do not use reagents beyond the kit expiration date.
10. DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
11. Incubate at room temperature for fifteen (15) minutes.

Results:

1. Perform a control of the system using the appropriate control to determine the assay performance.
2. Plot the absorbance for each duplicate serum reference versus the corresponding cTnI concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw a smooth curve through the plotted points.
4. To determine the concentration of cTnI for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph. (The duplicates of the unknown may be averaged as indicated). In the following example, the

Data and graphs are not visible in the text.
average absorbance (0.322) intersects the dose response curve at 3.61 ng/ml cTnI concentration (See Figure 1).  

**Note:** Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validated result should be used in lieu of a dose response curve prepared with each assay.

### Example 1

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Well Number</th>
<th>Abs (A)</th>
<th>Mean Abs (B)</th>
<th>Value (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cal A</td>
<td>A1</td>
<td>0.007</td>
<td>0.007</td>
<td>0</td>
</tr>
<tr>
<td>Cal B</td>
<td>C1</td>
<td>0.035</td>
<td>0.037</td>
<td>0.4</td>
</tr>
<tr>
<td>Cal C</td>
<td>E1</td>
<td>0.140</td>
<td>0.118</td>
<td>1.25</td>
</tr>
<tr>
<td>Cal E</td>
<td>A2</td>
<td>0.777</td>
<td>0.777</td>
<td>7.5</td>
</tr>
<tr>
<td>Cal F</td>
<td>B2</td>
<td>0.776</td>
<td>0.777</td>
<td>7.5</td>
</tr>
<tr>
<td>Ctrl 1</td>
<td>F2</td>
<td>0.051</td>
<td>0.051</td>
<td>0.43</td>
</tr>
<tr>
<td>Ctrl 2</td>
<td>A2</td>
<td>1.256</td>
<td>1.245</td>
<td>12.75</td>
</tr>
<tr>
<td>Patient</td>
<td>B3</td>
<td>0.324</td>
<td>0.322</td>
<td>3.61</td>
</tr>
</tbody>
</table>

*The data presented in Example 1 and Figure 1 is for illustration only and should not be used in lieu of a dose response curve prepared with each assay.*

#### 11.0 QC PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator A’ should be ≤ 0.07.
2. The absorbance (OD) of calibrator A’ should be ≥ 0.1.
3. Four out of six quality control pools should be within the established ranges.

#### 12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product is available on request from Monobind Inc.

### 12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) mm to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. 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-duration during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spuriously results.

### 13.0 EXPECTED VALUES

Troponin-I values are different in plasma and serum. In addition, plasma samples may be influenced by the additives used. For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient’s history and, all other clinical findings.

#### 13.1 Initial Screening

- **Normal Adult**: ≤ 0.04 ng/ml
- **Normal Elderly**: ≤ 0.04 ng/ml

### 13.2 Interpretation

Measurement and interpretation of results must be performed by a skilled individual or trained professional.

1. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy.
2. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
3. If test and standard are prepared from different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.

#### 13.3 Intra-assay and Inter-assay Variability

- **Intra-assay**: 3.6% and 5.5%
- **Inter-assay**: 6.6% and 2.0%

#### 13.4 Accuracy

For in vitro diagnostic use only.

#### 13.5 Specificity

For in vitro diagnostic use only.

#### 13.6 Sensitivity

For in vitro diagnostic use only.

### 14.0 PERFORMANCE CHARACTERISTICS

**14.1 Precision**

The within- and between assay precision of the cTnI AccuBind® ELISA Test has been determined by analyses on three different levels of pool control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

**14.2 Linearity**

The presence of lipemia (25 mg/dl); hemoglobin (4.0 mg/dl) and bilirubin (2.5 mg/dl) did not affect the assay precision.

**14.3 Accuracy**

The mean (± SD) cTnI AccuBind® ELISA Test System was compared with a predicate radioimmunoassay assay. Biological specimens from population (symptomatic and asymptomatic) were used. The (values ranged from N0 = -1.0 ng/ml) The total number of such specimens was 151. The data obtained is displayed in Table 4.

### 15.0 REFERENCES


### 16.0 Effective Data: 2019-Jan-01 Rev. 4

**DCO: 1320 MP3825 Product Code: 3825-300**

For orders and inquiries, please contact us.